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THE AMERICAN
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THE EXCRETION OF INULIN BY THE DOG

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The present paper reports observations on the excretion of the polysaccharid inulin by the normal and phlorizinized dog, these observations having been made prior to, or concurrently with, the observations on man recently reported by Shannon and Smith (1935). The introduction of inulin in renal function studies has been discussed by Richards, Westfall and Bott (1934), by Shannon (1934), by Professor Richards in his 1935 Harvey Lecture, and by Shannon and Smith in the above paper, and this discussion need not be extended here.

CHEMICAL METHODS. The chemical methods used in this investigation were the same as those described by Shannon and Smith (1935), except that in some of the earlier experiments sugars were determined both on a copper sulphate-sodium tungstate filtrate (Somogyi, 1931) by the Folin (1929) sugar method, as well as on the iron filtrate of Steiner, Urban and West (1932) by the Shaffer-Somogyi (1932) method. Extensive observations on recovery of inulin, glucose and xylose from plasma and urine have been made with both methods. The Folin method gives incomplete reduction with xylose but when plasma and urine are handled alike one should obtain the same U/P ratio by this as by the Shaffer-Somogyi method, especially since the saccharoid blank is low with both methods of precipitation (2 to 3 mgm. per cent with the copper and 1 to 2 mgm. per cent with the iron). Nevertheless, in experiments where both methods have been used, it has been observed that the Shaffer-Somogyi method gave values for the xylose clearance averaging five per cent lower than by the Folin method, although the inulin and glucose clearances by the two methods were identical. The reason for this discrepancy is not revealed

[1] This paper is based on a thesis to be presented to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by recoveries. All data recorded here were obtained by the Shaffer-Somogyi method on an iron filtrate. White and Monaghan (1933), using this technique, were unable to obtain the nearly exact correspondences between the creatinine, glucose and xylose clearances previously reported from this laboratory for the phlorizinized dog. We have been unable to confirm these investigators, however, and continue to secure this correspondence, except as noted above.

EXPERIMENTAL PROCEDURE. Normal female dogs weighing from 15 to 18 kgm. were used in all our experiments. A 20 per cent solution of inulin in 0.6 per cent saline, prepared at 85°C., was administered either intravenously, subcutaneously, or by both routes. One does not obtain by the subcutaneous route plasma concentrations higher than 100 mgm. per cent even when fairly large doses are given, and for this reason the inulin was given in most of our experiments intravenously in doses of from 2 to 4

TABLE 1

Showing the relationship existing between plasma level and rate of excretion of inulin

DOG D			DOG 8		
Plasma	Urine	Clearance	Plasma	Urine	Clearance
mgm. per cent	*mgm. per min.*	*cc. per min.*	*mgm. per cent*	*mgm. per min.*	*cc. per min.*
565	350	62.0	309	108.8	35.2
380	237	62.4	227	82.6	36.4
230	139	60.4	164	59.2	36.1
143.5	92.2	64.2	120	42.0	35.0
86	53.3	62.0	88.2	32.0	36.2
53.5	32.8	61.4	63.8	22.8	35.8

grams per kilogram, or in doses of from 1 to 2 grams per kilogram subcutaneously followed by a like amount intravenously.

Xylose was administered by stomach, creatinine subcutaneously, and phlorizin intravenously or by a combination of subcutaneous and intravenous injections. The conduct of the experiments was on the whole identical with those previously reported.

RESULTS. The experiments reported in table 1 show that the rate of excretion of inulin in the dog is directly proportional to the plasma concentration between values of 53 and 565 mgm. per cent. It follows from this fact that the inulin clearance is independent of its plasma level. The curves generated by plotting plasma level against rate of excretion extrapolate through the zero coördinates, indicating that the relationship holds true at very low as well as at the observed plasma levels.

An experiment showing that the administration of inulin intravenously does not affect the clearances of urea, xylose or creatinine, either absolutely

TABLE 2

Data to show that the intravenous infusion of inulin has no effect on the renal clearances of other substances

PERIOD NUMBER	URINE FLOW	PLASMA				CLEARANCES				CLEARANCE RATIOS					
		Urea	Xylose	Inulin	Creatinine	Urea	Xylose	Inulin	Creatinine	$\frac{\text{Urea}}{\text{Xylose}}$	$\frac{\text{Creatinine}}{\text{Xylose}}$	$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$	
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	*cc. per min.*	*cc. per min.*						
1	2.53	12.4	80.5		24.6	21.9	29.5		41.1	0.74	1.39				
2	2.10	12.5	80.7		27.6	19.0	25.4		38.0	0.75	1.50				
3*	1.83	12.6	81.7		29.4	19.1	26.0		38.6	0.74	1.48				
Average						20.0	27.0		39.2	0.74	1.46				
4	2.00	12.6	81.8	139	37.0	18.4	23.4	35.0	34.4	0.78	1.47	0.53	0.67	0.98	
5	1.53	12.9	77.6	101	37.4	20.8	28.5	41.6	40.9	0.72	1.44	0.50	0.69	0.98	
6	0.88	13.3	72.6	78	36.8	20.6	28.6	41.5	41.3	0.72	1.44	0.50	0.69	1.00	
Average						19.9	26.8	39.4	38.8	0.74	1.45	0.51	0.68	0.99	

* At the conclusion of period 3, 1.5 gram per kilogram of inulin in 0.6 per cent NaCl was given by slow intravenous infusion. Five-tenths gram xylose with 20 cc. water per kilogram were given by stomach, and 0.2 gram creatinine per kilogram in water were given subcutaneously. Period 4 started 60 minutes after end of period 3.

TABLE 3

Comparison of xylose, inulin, and creatinine clearances in the normal dog

Xylose given by stomach tube, creatinine and inulin by subcutaneous injection. Dog 9, January 18, 1934.

PERIOD NUMBER	URINE FLOW	PLASMA			CLEARANCE			CLEARANCE RATIOS	
		Xylose	Inulin	Creatinine	Xylose	Inulin	Creatinine	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	*cc. per min.*		
1	3.6	50.3	104	13.7	35.0	48.6	49.1	0.72	1.01
2	3.33	51.6	106	14.7	34.8	47.7	49.1	0.71	1.03
3	3.13	53.1	108	16.0	36.0	50.7	50.1	0.72	0.99
4	2.87	54.0	109	16.6	33.9	47.1	47.6	0.72	1.01
Average								0.72	1.01

or relative to each other, is given in table 2. This point is an essential one in the administration of any substance for renal function studies.

The creatinine clearance is identical with that of inulin under all conditions that we have examined. A typical experiment illustrating simultaneous creatinine and inulin clearances is given in table 3, and data from the entire series are given in figure 1. The mean of 42 comparisons on 8 dogs gives a creatinine/inulin ratio of 0.994 with a standard deviation of 0.034, and maximum variations of +0.086 and −0.064. In these experi-

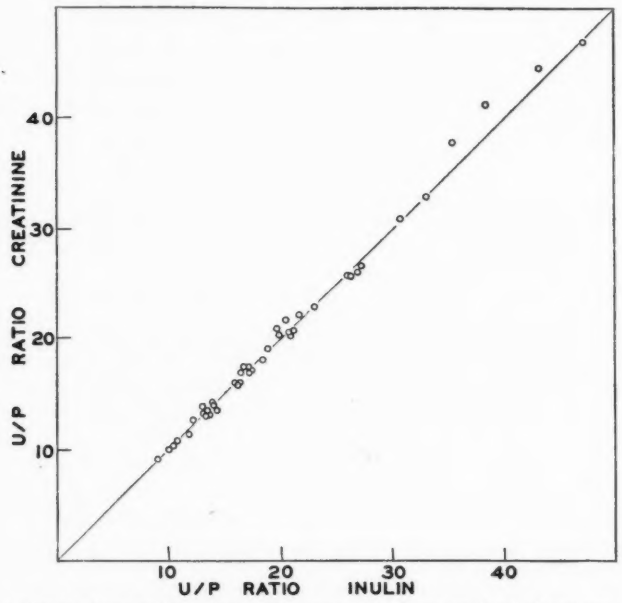

Fig. 1. Data showing that the simultaneous U/P ratios (and therefore the clearances) of creatinine and inulin are equal in the normal dog. This equality is maintained after phlorizin.

ments the plasma creatinine varied from 6.0 to 130 mgm. per cent, and the U/P ratio of inulin from 9 to 47. Lack of space precludes the presentation of the data in full, but it may be commented that we find the creatinine clearance to be independent of the plasma level of this substance, as is the case with inulin.

The xylose clearance in the normal dog is less than the simultaneous inulin clearance. In this finding we confirm Richards, Westfall and Bott (1934), whose data, however, show a wider deviation between the two clearances than ours do. A series of 24 observations on 6 dogs is illustrated in figure 2. The mean xylose/inulin ratio in these data is 0.734

and the standard deviation of the mean is 0.022. All these observations were made at urine flows above 0.88 cc. per minute, but with inulin U/P ratios ranging from 11.1 to 76.2. A larger series of observations on this point was not considered necessary since the creatinine clearance has been shown in a number of previous papers to exceed the xylose clearance by about this amount.

Data from a typical experiment illustrating the action of phlorizin are given in table 4, and a summary of observations of 5 dogs before and after phlorizin (30 periods in all) is given in table 5. Since phlorizin depresses all renal clearances it is necessary in making comparisons before and after the administration of this drug to take one clearance as a standard of refer-

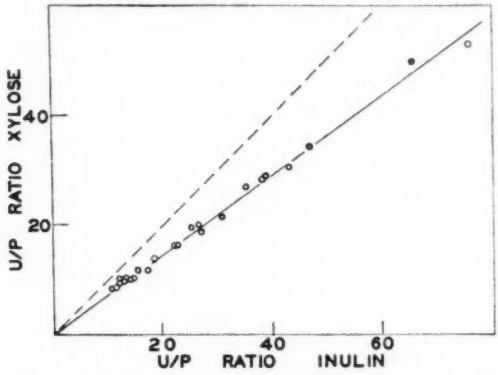

Fig. 2. Data showing that the U/P ratio (and therefore the clearances) of xylose in the normal dog is less than the simultaneous U/P ratio of inulin. The dotted line represents a xylose/inulin ratio of 1.0, and the solid line a ratio of 0.734, the mean of the observations. Phlorizin raises the xylose clearance to within 10 per cent of the inulin clearance.

ence. In tables 4 and 5 we have chosen the inulin clearance for this purpose. The administration of phlorizin has no effect upon the relative values of simultaneous inulin and creatinine clearances, these remaining identical within the limits of experimental error. Glucose, of course, appears in the urine after phlorizin, the clearance of this substance being identical with that of creatinine, as previously reported by Shannon, Jolliffe and Smith (1932), and therefore identical also with the inulin clearance. After phlorizin the xylose clearance rises relative to the inulin clearance, but does not come to equal it, remaining about 10 per cent below the latter. (Comparisons made by the Folin sugar method on a copper tungstate filtrate give xylose clearances about 5 per cent higher, as reported under Methods; in view of this fact the xylose clearance may really be within 5 per cent of the inulin clearance in the phlorizinized ani-

TABLE 4

Comparison of urea, xylose, glucose, inulin and creatinine clearances before and after phlorizin (100 mgm. per kgm. intravenously)

Dog 8. February 15, 1934.

PERIOD NUMBER	URINE FLOW	PLASMA					CLEARANCE					CLEARANCE RATIOS			
		Urea	Xylose	Glucose	Inulin	Creatinine	Urea	Xylose	Glucose	Inulin	Creatinine	$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$
	cc. per min.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per min.*	*cc. per min.*	*cc. per min.*	*cc. per min.*	*cc. per min.*				
1	2.30	11.5	75.0		174	30	20.1	27.3		37.2	36.6	0.54	0.73		0.98
2	1.65	11.6	73.4		139	32	19.8	26.9		38.0	38.0	0.52	0.71		1.00
3	1.20	11.7	66.8		114	33.5	19.8	25.9		36.7	37.2	0.54	0.71		1.01
Average												0.53	0.72		1.00
4*	1.20	11.7	54.0	147	86.7	35.9	15.2	23.2		25.0	24.5	0.61	0.93		0.98
5	1.20	11.7	49.0	139	79.3	34.9	14.4	22.0	24.8	25.0	24.5	0.58	0.88	0.99	0.98
6	1.14	11.7	41.6	131	67.0	34.1	14.8	22.8	26.6	26.0	26.0	0.57	0.88	1.02	1.00
Average												0.59	0.90	1.01	0.99

* Following Phlorizin, 100 mgm. per kilogram, intravenously.

TABLE 5

Summary of comparisons of urea, xylose, inulin, creatinine and glucose clearances before and after phlorizin

ANIMAL NUMBER	BEFORE PHLORIZIN					AFTER PHLORIZIN						
	Number of periods	Inulin clearance*	Clearance ratios			Dose of phlorizin		Number of periods	Change in inulin clearance*	Ratio of clearances*			
			$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$	Intravenously	Subcutaneously			$\frac{\text{Urea}}{\text{Inulin}}$	$\frac{\text{Xylose}}{\text{Inulin}}$	$\frac{\text{Creatinine}}{\text{Inulin}}$	$\frac{\text{Glucose}}{\text{Inulin}}$
		cc. per min.				*mgm. per kilo*	*mgm. per kilo*		*per cent*				
4	3	35.5			0.98	200		3	−19.0			0.98	0.97
5	3	46.6			0.99	100	100	3	−14.0			0.99	0.99
7	3	72.2		0.77	0.99	100	100	3	−44.0		0.93	0.96	1.07
8	3	37.3	0.53	0.72	1.00	100		3	−31.0	0.59	0.90	0.99	1.01
30	3	75.7	0.63	0.76	1.06	100		3	−31.0	0.64	0.90	1.02	1.02
Average			0.58	0.75	1.00				−28.0	0.61	0.91	0.98	1.01

* Average of all periods.

mal.) In a limited series of experiments no significant change has been observed in the urea/inulin ratio before and after phlorizin.

The excretion of sucrose relative to inulin has not been extensively examined. In those experiments where this substance was present the results were in accord with the relationship reported here between xylose and inulin.

Discussion. The only independent evidence that can be advanced that inulin is not excreted in part by tubular secretion in the normal dog, is the fact that its clearance is independent of the concentration in the plasma, between wide limits of the latter. Since this evidence is not absolutely exclusive, the presumption against secretion must rest upon the evidence obtained from the aglomerular fish (Richards, Westfall and Bott, 1934; Shannon, 1934) and the behavior of carbohydrates in general, as discussed by Shannon and Smith (1935). It is significant that in the dog the creatinine clearance is independent of the plasma level of this substance, as has previously been pointed out from this laboratory, and confirmed in the experiments described in this paper. The behavior of this substance in the dog is quite different from what it is in man, where a markedly curvilinear relationship is observed (Shannon, 1935). Granted that a linear relationship between plasma level and rate of excretion is, in these two instances, evidence against secretion of either substance, it is a confirmation of this evidence that the simultaneous inulin and creatinine clearances are equal, within the experimental error of observation, in both the normal and phlorizinized dog.

It follows from the above interpretation regarding the non-secretion of inulin that some xylose is reabsorbed in the normal dog, the figures presented here indicating the fraction to be about 27 per cent of that which is filtered. The fact that the xylose/inulin ratio does not vary with variation in inulin U/P from 11.1 to 76.2 might be taken to indicate that the degree of concentration of the urine is not the predominating factor in the discrepancy between the excretion rates of these two substances.

Since phlorizin raises the xylose clearance relative to the inulin clearance, it must be inferred that reabsorption in the dog, as in the dogfish and man, is in part an active process. In the three experiments on the phlorizinized dog reported here, the xylose clearance is still 10 per cent below the inulin clearance, a difference that is only 5 per cent when the Folin method, instead of the Shaffer-Somogyi method, is used. In view of the much larger series of comparisons previously published from this laboratory (in which the Folin sugar method and copper tungstate filtrate were used), showing almost exact agreement in the clearances of these substances in the phlorizinized animal, the significance of this difference is questionable.

Shannon, Jolliffe and Smith (1932) based their conclusion that creati-

nine was secreted in the normal dog on the facts that: *a*, the creatinine clearance exceeds the simultaneous xylose and sucrose clearances; *b*, under phlorizin, these clearances come to equal each other, an equalization that is effected by a fall in creatinine clearance rather than a rise in xylose clearance; and *c*, phlorizin does not change the urea/xylose ratio, as might be expected if the drug were specifically blocking the reabsorption of the pentose. Pitts (1934) in a subsequent examination of the excretion of creatine, confirmed the first two observations, noting that on the administration of phlorizin, the creatine and creatinine clearances dropped to approximately the same level, the glucose rose to the xylose level, and the xylose clearance itself was not appreciably changed. The constancy of the xylose clearance before and after phlorizin may, however, be an artifact due to a decrease in glomerular filtration equal to a diminished reabsorption of the sugar. The fact that in Pitts' (1934) experiments the average creatine/creatinine ratio remained constant (0.89 before and 0.92 after phlorizin) in spite of a fall in the absolute value of both clearances, whereas the ratio of the xylose clearance to the other two rose, points in this direction. The apparent constancy of the urea/xylose ratio before and after phlorizin, which Jolliffe, Shannon and Smith (1932) emphasized as evidence against reabsorption of xylose, has also been noted in man (Chasis, Jolliffe and Smith, 1933) but, as Shannon and Smith (1935) have pointed out, this ratio is now known to change with changes in the absolute level of these clearances, and such independent changes may partly or wholly occlude the change due to the blockage of the reabsorption of the sugar.

It would appear from the evidence obtained by the use of insulin that xylose is actively reabsorbed in the normal dog, and that the difference between the xylose and creatinine clearances is due to this reabsorption. The extent of this reabsorption, as judged from the inulin clearance, appears to be about the same in the dog as in the dogfish and man.

When we consider all the evidence available on the dogfish, man, and dog, we are led to the following interpretation: that inulin is not secreted by any of these and, assuming no reabsorption of this substance (*vide infra*), its clearance is very close to, if not identical with, the rate of glomerular infiltration. The capacity to secrete creatinine, evident in the dogfish and man, is vestigial or absent in the dog: hence the equality of the inulin and creatinine clearances. This equality is not perturbed by phlorizin, and since phlorizin brings the glucose clearance up to the inulin and creatinine clearances, it appears that this drug completely blocks the reabsorption of glucose in the renal tubules, as was believed by Jolliffe, Shannon and Smith (1932).

Since an apparent active reabsorption of some xylose has been uncovered in these experiments, it seems possible that there might also be an active reabsorption of some inulin. No evidence bearing on this question can be

advanced at the present time, unless it be the mere fact of the identity of the creatinine and inulin clearances in the normal animal.

With regard to the passive reabsorption (diffusion) of inulin, it will be noted that the equality between the creatinine and inulin clearances holds at U/P ratios of inulin varying from 9 to 47, and that in the phlorizinized dog this equality also obtains with the simultaneous glucose clearance. It has been shown that the simultaneous xylose and sucrose clearances in the normal dog, and xylose and glucose clearances in the phlorizinized dog, are equal (Jolliffe, Shannon and Smith, 1932; Pitts, 1934); it appears from these facts that the differential diffusion of these substances must be very small, and that the diffusion of a molecule as large as inulin (mol. wgt. 972 or greater) is probably negligible. But this argument cannot be extended to the abnormal kidney, or to the normal kidney at very low urine flows, without the support of further evidence.

SUMMARY

The rate of inulin excretion in the dog is directly proportional to plasma concentration between values of 53 and 565 mgm. per cent.

The intravenous administration of inulin does not affect the urea, xylose or creatinine clearances.

In a series of 42 comparisons on 8 dogs, the ratio of the simultaneous creatinine and inulin clearances has a mean value of 0.994 with a standard deviation of 0.034, and maximum variations of +0.086 and −0.064.

The xylose clearance in the normal dog at moderate to high urine flows is less than the simultaneous inulin clearance, the mean xylose/inulin ratio being 0.734, with a standard deviation of 0.022.

Under phlorizin the glucose clearance rises to the level of the creatinine and inulin clearances, the equality of which is maintained, while the xylose clearance is raised to within 10 per cent of the inulin clearance.

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THE EFFECT OF ONE BORDER IN THE VISUAL FIELD UPON THE THRESHOLD OF ANOTHER

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Two types of test objects are widely used for measuring brightness discrimination. One consists of a disk on a slightly darker field. In this case the capacity to discriminate brightness is measured by the minimum brightness difference between the area and its background which is just perceptible. This is called the brightness difference threshold. Although it is generally spoken of as the threshold of the area, it may be regarded as the threshold of the border, and this way of conceiving the threshold will be employed in the present paper.

The second type of test object used for measuring brightness discrimination is a bisected object, one half of which is slightly darker than the other. The threshold in this case is the brightness difference between the two halves necessary for the border between them to be just perceptible.

In the present paper we shall refer to the border for measuring the threshold as the *test* border. Other borders in the vicinity which affect the threshold will be called activating borders. In order to give the reader a clue for understanding and relating the numerous phenomena to be described, it may be pointed out here that whenever an activating border acts on the side of a test border, as must always be the case when the test border is continuous, the effect is invariably an interference with the establishment of the border which raises the threshold. Figure 2 presents a situation of this kind. When, as happens in the case of figure 7 where a bisected test-object is used, the activating border acts on the ends of the test border, it facilitates the establishment and thus lowers the threshold.

Although previous investigators have reported phenomena which are related to this problem, they have in some cases attributed the results to unessential factors in the stimulus conditions. In such cases it is necessary to analyze carefully the rôles played by the various factors. In the second place we have tried to tie together systematically a number of phenomena which have heretofore been treated in isolation from each other.

The first phenomenon to be considered was first described by Blachow-

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ski (1913). He used a stimulus pattern similar to figure 2 and showed that the differential threshold between the disk, A, and the annulus, B, decreases as the width of the annulus, B, increases. The results we have obtained are shown in figure 4. Blachowski attributed his phenomenon to the inclusion of more area within B. The inadequacy of this factor to account for the phenomenon can be shown by using a stimulus pattern like figure 3. The width of the black band and the outside diameter of C were kept constant so that the total area $(C + B)$ remained constant,[2] and the only factor varied was the distance of the black ring from A. The explanation of the decrease in threshold in this case seems to be that the border at the outer edge of B interferes with the establishment of the border at the edge of A, and the amount of interference decreases as the outer edge of B gets more and more remote from the edge of A. At a distance of $1\frac{7}{16}$ inches the interference becomes practically negligible. Since the same results are obtained with figure 2 as with figure 3, the decrease in the threshold may be attributed to a release from the interference effect of the outer edge of B rather than to the inclusion of more area within B.

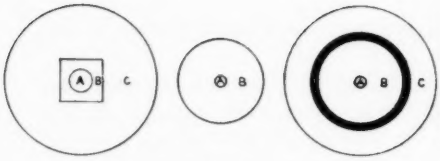
The pattern in figure 3 demonstrates a second fundamental property of a border, that of blocking activity from spreading across the retina. Since the same results are obtained with the annulus, C, present and absent, it must be assumed that the border at the outer edge of B blocks the border at the inner edge of C from affecting the threshold of A.

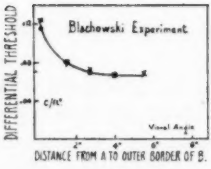
Blachowski's phenomenon is closely related to the fact that the threshold for an area on a slightly darker ground decreases as the size of the area increases. Although previous investigators have attributed this to the inclusion of more receptors within the area, evidence is presented below that the essential stimulus factor is the separation of the borders. In other words when a disk shaped area is used for measuring the threshold, the borders on opposite sides of the area tend to interfere with each other, so that, when the disk is small, the interference is great and the threshold high, but this interference dies out as the separation of the borders increases, and consequently the threshold falls.

We have measured the differential threshold for disk-shaped areas subtending visual angles ranging from 0.43° to 8.05°. The results are shown in figure 5. When these results are compared with the results of the Blachowski experiment shown in figure 4, it is seen that change in threshold dies out at the same separation of borders in both cases, namely, about 4°. This may be interpreted as evidence that the variation of area experiment as well as Blachowski's experiment involves the interference of one border by another.

The same type of thing comes into play in Dittmers' (1929) experiment,

[2] As the diameter of the ring increases its area also increases, thus changing the total area of $C + B$, but the change is so slight that it may be ignored.





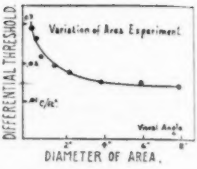


Fig. 1 Fig. 2 Fig. 3 Fig. 4 Fig. 5

Fig. 1. Pattern for demonstrating Dittmers' phenomena. C was $1\frac{1}{4}$ inches in diameter. B was $\frac{1}{2}$ inch on each side. A was $\frac{1}{4}$ inch in diameter. The distance from the stimulus to the eye was 38 inches. An artificial pupil 2.33 mm. in diameter was placed in front of the right eye. The left eye was shielded completely. The experiment was performed in a dark room with nothing visible but the stimulus pattern. The object of the experiment was to investigate the effect of varying the brightness of C upon the differential threshold for A.

Fig. 2. Pattern for demonstrating Blachowski's phenomenon. The diameter of A was $\frac{3}{8}$ inch. The brightness of B was set at 0.468 c. per sq. ft. An artificial pupil 2.33 mm. in diameter was placed before the right eye. The left eye was shielded completely. The stimulus was $28\frac{1}{2}$ inches from the eye. The object of the experiment was to demonstrate the effect of varying the size of B upon the differential threshold for A.

Fig. 3. Pattern for demonstrating the inadequacy of Blachowski's type of explanation. The diameter of A was $\frac{3}{8}$ inch. The outside diameter of C was 6 inches and the width of the black band was $\frac{1}{4}$ inch. The brightness of C and B was kept constant at 0.468 c. per sq. ft. Other conditions were the same as for the pattern in figure 2. The object of the experiment was to investigate the effect of varying the diameter of the black band upon the threshold for A, and to ascertain whether the presence of the annulus, C, has any effect upon the results as compared with the results obtained with the pattern in figure 2.

Fig. 4. An analysis of Blachowski's phenomenon as demonstrated with the patterns in figures 2 and 3. The crosses show the effect of varying the distance from A to the outer border of B in figure 2. The circles show the effect of varying the distance from A to the outer border of B in figure 3 with the width of the dark ring and the outside diameter of C kept constant. The differential threshold specifies the brightness difference between A and B necessary for A to be just perceptible. In these experiments A was always brighter than B. Fry served as subject.

A series of five measurements of the threshold for A was made for each size of B for both patterns. The series for each size of B for figure 2 was followed by a series for figure 3 with B of the same size. In this way readings for the two different patterns for a given size of B were made with the condition of the eye practically unchanged. The different sizes of B were investigated in the order of increasing size. The experiment was repeated in reverse order and the results were averaged. Hence each value in the graph in figure 4 represents an average of 10 readings. At the beginning of the experiment and between each pair of series dealing with a given size of B, a series of readings was made with figure 3 from which the black band was removed. This pattern served as a standard for measuring changes in the general condition of the eye. The threshold with this pattern remained practically unchanged during the course of the experiment, showing an average value of 0.07 c. per sq. ft.

Fig. 5. The effect upon the brightness difference threshold of varying the diameter of an area. Areas of different sizes were presented on a bright background (0.9 c. per sq. ft.) which was 16 inches by 20 inches and 20.75 inches from the eye. Two readings were made for each area, one series being made in the order of increasing size and the other in the order of decreasing size. Bartley served as subject, using one eye without an artificial pupil.

which can be demonstrated with the pattern in figure 1. The object of the experiment is to investigate the effect of varying the brightness of C upon the differential threshold for A. We have performed two separate experiments, one in which A was brighter than B and another in which A was darker than B. The results are shown in figure 6. In both cases the mini-

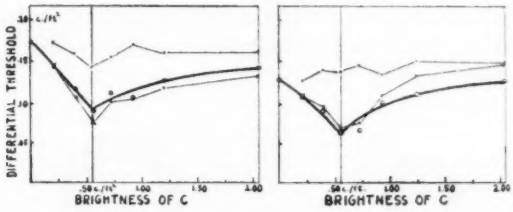


Fig. 6. These graphs show the results obtained with the stimulus pattern in figure 1. The object of the experiment was to investigate the effect of varying the brightness of C upon the differential threshold for A, i.e., the brightness difference between A and B necessary for A to be just perceptible. In the left hand graph A was brighter than B; B was kept constant at 0.565 c. per sq. ft. (the level indicated by the vertical line), and A was varied to measure the threshold. In the right hand graph A was darker than B; A was kept constant and B varied.

In each experiment the measurements were all made at one sitting. The different levels of brightness of the surrounding field were taken in the order of increasing brightness. Alternate readings were made with the surrounding field present and absent. Five such pairs of readings were made at each level. The values given in the graph represent therefore averages of five readings. When the readings were alternated with the surrounding field present and absent, changes in the condition of the eye could be detected, if any occurred during the experiment, by the change in the threshold with the surrounding field absent. The empirical values obtained with the surrounding field present can be corrected for changes in the condition of the eye by determining the ratio between the value for "field present" and "field absent" and multiplying the original value for "field absent" by this ratio. These corrected values give a truer expression of the phenomenon than the raw empirical data. In each graph the line through the dots represents the change in the general condition of the eye during the course of the experiment as measured by the differential threshold between A and B when C was zero. The crosses represent the absolute values obtained for the different brightness levels of C, and the circles represent the corrected values as explained above. Fry served as subject in these experiments.

mal threshold value was obtained when B and C were equal in brightness; the threshold rises when C is either higher or lower than B.

Dittmers suggested that contrast which maintains the border between B and C is the factor responsible for the behavior of the threshold, and in accordance with this explanation the interpretation of the salient facts may be summarized as follows: 1. When the surrounding field, C, is either darker or brighter than zone B the resulting contrast border interferes with the establishment of a border at the edge of A and hence raises the thresh-

old for A. 2. As the brightness of B and C approach equality, contrast between them is lessened, and the border of A is gradually released from interference, and as a result the threshold falls. 3. It does not matter which of the two sides of the activating border is dark or bright, it has the same qualitative effect upon the border, one of interference. 4. Nor does it matter whether A is darker or brighter than B, the effect upon the border between them is still one of interference. 5. Since the border between A and B is a circle, the activating border acts sidewise against it, because there is no end to be acted upon.

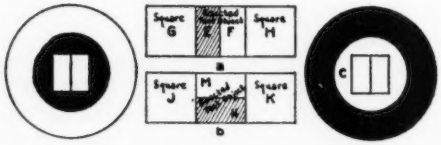


Fig. 7 Fig. 8 Fig. 9

Fig. 7. Pattern for demonstrating the effect of the dark side of an activating border upon the ends of a test border. The thickness of the ring was $\frac{3}{8}$ inch. The inside diameter was 1 inch. The bisected test object was $\frac{1}{2}$ inch on each side. The brightness of the dark half of the test object was set at 0.565 c. per sq. ft. and that of the bright half was varied to measure the threshold. The effect upon the threshold of varying the brightness of the ring was investigated. The distance to the eye was 38 inches. The left eye was shielded and an artificial pupil 2.33 mm. in diameter was placed before the right eye.

Fig. 8. Patterns for analyzing the effects of varying the brightness of C in figure 9. In each pattern the squares as well as the bisected test object were $\frac{1}{2}$ inch on each side. The brightness of the dark half of the test object was set at 0.565 per sq. ft. and the bright half was varied to measure the threshold. The effect upon the threshold of varying the brightness of the squares was investigated. The distance to the eye was 38 inches. The left eye was shielded and an artificial pupil 2.33 mm. in diameter was placed before the right eye.

Fig. 9. Pattern for demonstrating the effect of the bright side of an activating border upon the ends of a test border.

Geldard's phenomenon (Geldard, 1931; Bartley and Fry, 1934), demonstrates the effect of an activating border upon the end of a test border. The phenomenon can be demonstrated with a pattern like figure 7, although in Geldard's original pattern a bright disk to one side of the test object was used instead of a ring completely surrounding it. The inside border of the white ring acts upon the ends of the bisecting border, but is prevented from acting upon the sides of the bisecting border by the borders which separate the two halves of the test object from the surrounding field. It is impossible to demonstrate this by any manipulation of figure 7, but it is quite possible under more adequate circumstances to demonstrate that one border can block the activity spreading from another, and there seems

to be no reason why the same principles should not hold in the case in question. An instance of the blocking of the effects of one border by another is found in the case of figure 3, for the outer border of B blocks the inner border of C from affecting A.

The effect of varying the brightness of the ring in figure 7 upon the threshold of the bisecting border is shown in the left hand graph in figure 10. As the brightness of the ring increases the threshold of the bisecting border decreases. The interpretation of this is that when an activating border acts upon the ends of a test border it lowers its threshold.

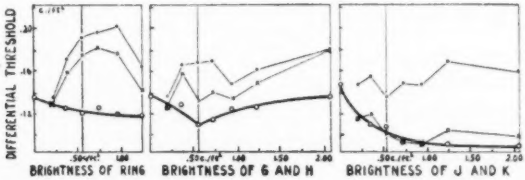


Fig. 10. The left hand graph shows the effect of the dark side of a border upon the end of a test border as demonstrated by varying the brightness of the ring in the pattern in figure 7.

The center graph shows the rôle played by borders at the sides of a bisected test object as demonstrated by varying the brightness of the two squares, G and H, in figure 8a.

The right hand graph shows the effect of the bright side of a border upon the ends of a test border as demonstrated by varying the brightness of J and K in the pattern in figure 8b.

The differential threshold specifies the difference in brightness between the two halves of the test object necessary for a perceptible border. The brightness level of the dark half of the test object is indicated in each graph by the vertical line. In each graph the line through the dots represents the change in the sensitivity of the eye during the course of the experiment as measured by the threshold for the test object alone in a dark field. The crosses represent the absolute values obtained for the different conditions, and the circles represent the corrected values as explained in the legend of figure 6. The same procedure as to order of readings has been used in these experiments as described in the legend of figure 6. Fry served as subject.

Figure 9 is strictly analogous to figure 7 but involves a reversal of brightness relationships. The pattern in figure 9 is the most difficult to deal with of any that we have yet considered. The effects obtained from varying the brightness of zone C are confusing, because this stimulus variable manipulates the borders both at the outer edge of C and at the juncture between C and the test object. It is highly desirable to demonstrate the effects of these borders separately and to this end the two patterns in figure 8 have been designed. In both patterns the effect of varying the brightness of the squares was investigated. The results are given in the center and the right hand graph in figure 10.

The experiment with figure 8a is simply a modification of Dittmers'

experiment. The effect of the border between G and E upon the bisecting border is analogous to the effect of the border between B and C in figure 1 upon the border at the edge of A when A is brighter than B. Likewise the effect of the border between H and F upon the bisecting border is analogous to the effect of the border between B and C in figure 1 upon the border at the edge of A when A is darker than B. Therefore on the basis of the results obtained with figure 1 we should expect that the borders between E and G and between F and H would interfere with the establishment of the bisecting border when G and H are above or below E and F in brightness, and that this interference would decrease as G and H approach the brightness level of E and F. This is exactly what happens.

The same type of phenomenon as that just described has been demonstrated in an experiment by Cobb (1916) who investigated the effect of varying the brightness of a field surrounding a bisected test object, but instead of using a field of limited size and definitely bounded like figure 9, Cobb included the whole field of vision. In this situation the only borders which can act upon the bisecting border are those which separate the two halves of test object from the surrounding field. These borders tend to raise the threshold for the bisecting order, and hence the threshold should be higher when the surrounding field is above or below the level of the test object than when it is equal. Cobb obtained just such results.

The right hand graph in figure 10 shows that when the brightness of J and K in figure 8b is increased the threshold for the bisecting border continues to fall and does not show a subsequent increase when the brightness of J and K exceeds the level of M and N. The explanation of this phenomenon seems to be that the extreme borders of J and K set up activities which spread across the squares and act on the ends of the bisecting border, lowering its threshold. But the possibility has not been eliminated that the effect is mediated by changes at the inner borders of the squares, although it seems that if this were the case a reversal would have occurred as the brightness level of the squares passed by that of the test object.

A good method for investigating this problem would be to leave the brightness of C constant in figure 9 and investigate the effect of varying the diameter of C. The nearer the outer edge of C to the test object, the lower should be the threshold. Our apparatus was not adapted to the problem, however.

SUMMARY

Experiments have been devised to study the effect of one border in the visual field upon the threshold of a neighboring border. When the activating border acts on the sides of the test border it tends to raise its threshold; when it acts on its ends it tends to lower its threshold. The activity which spreads from one border to another can be blocked by interposing a

third border between them. We are indebted to Drs. G. H. Bishop and P. W. Cobb for their criticisms and suggestions.

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REFLEX RESPONSES OF THE NICTITATING MEMBRANE

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Reflex activity of the nictitating membrane (n.m.) of the cat may involve either the extrinsic or the intrinsic muscles. In the former case, protrusion of the n.m. is obtained on mechanical stimulation of the cornea. The muscles active in this reflex are skeletal, chiefly the external rectus (Rosenblueth and Bard, 1932). The intrinsic musculature of the n.m., however, is smooth. It is supplied exclusively by sympathetic nerves (Rosenblueth and Bard, *loc. cit.*), and is responsible for the usual state of tonic contraction of the n.m. It is possible under appropriate experimental conditions, to be described, to obtain reflex contraction (central excitation) and relaxation (central inhibition) of the intrinsic musculature. The present paper deals with such reflexes. Participation of skeletal muscle was excluded by injecting curare in control experiments.

METHOD. Cats were used. They were anesthetized with ether and the femoral vein was exposed. A cannula was inserted into the trachea. The ether was then discontinued and urethane (1.5 gm. per kgm.) was slowly injected intravenously.

In all experiments the adrenal glands were exposed through a midline abdominal incision and were tied off. In some cases the vagi were severed in the neck and the carotid-sinus nerves were cut at their origin in the arteries.

The movements of the right n.m. were recorded isotonically by means of a light lever, after fixation of the head with a Czermak holder. The blood pressure (b.p.) was recorded from a carotid or a femoral artery by a mercury or a calibrated membrane manometer (Hürthle).

The nerves employed as afferents were cut peripherally, and buried shielded electrodes were applied to them. The stimuli were induction shocks at tetanizing frequency from a Harvard inductorium, or condenser discharges of variable capacities and intensities and at frequencies regulated by a metronome, or, finally, rectangular waves of variable intensities, durations and frequencies from a "multivibrator" circuit.

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RESULTS. A. *Reflex contraction.* The n.m. contracts readily after stimulation of all the afferent nerves thus far tried (Rosenblueth and Acheson, in preparation): cutaneous afferents (e.g., the saphenous), muscular afferents (e.g., the hamstring nerves), mixed somatic afferents (e.g., the sciatic) and visceral afferents (e.g., the splanchnics). In the present report we shall mainly describe observations in which the sciatic was used as an excitatory afferent. The general statements may be made that higher intensities and higher frequencies (up to 100 per second) of stimulation are more efficient in eliciting reflex contractions than lower intensities or frequencies and that the responses are a function of the intensity (spatial summation) or frequency (temporal summation), but these statements require qualifications which will be reported in later studies.

Single maximal shocks applied to the sciatic may evoke detectable reflex responses. As a rule, however, especially if smaller afferent nerves are employed—e.g., the saphenous—single afferent volleys are insufficient, and to evoke reflexes repetitive stimulation is necessary.

B. *Tonic contraction.* In the experimental conditions described the innervated n.m. is persistently semi-contracted as a consequence of a continuous tonic discharge of nerve impulses from the cervical sympathetic. If this nerve is severed the membrane relaxes. The degree of tonic contraction is usually moderate, about $\frac{1}{8}$ to $\frac{1}{4}$ of the maximal contraction of which the n.m. is capable. Not infrequently there are slow minor changes in the degree of tonic contraction. One of the factors responsible for these changes may be the depth of anesthesia; as a rule the tone was greater in lighter than in deeper anesthesia.

C. *Reflex relaxation.* Central inhibition of the tonic discharge described in the preceding section leads to a relaxation of the n.m. It is not easy to obtain from the afferents mentioned in section A, which lead to contraction; but it can be obtained in certain instances, of which figure 1A is an example. Low intensities and frequencies of stimulation are indispensable for inhibitory effects from these nerves.

Stimulation of the vagi (including the depressor fibers), on the other hand, readily leads to central inhibition and corresponding relaxation of the n.m. The effect may appear as a lessening of the tonic contraction (fig. 1B), as a depression of reflex contraction (fig. 2), or, finally, as a shortening of the after-discharge of a reflex contraction (fig. 3).

D. *The influence of the circulatory proprioceptors.* The ease with which afferent stimulation of the vagus elicits central inhibition (figs. 1, 2 and 3) suggested that its depressor component might be responsible. This idea further suggested that other circulatory proprioceptor afferents, e.g., the carotid-sinus nerves, might likewise inhibit the n.m. Afferent stimulation of the sciatic capable of eliciting reflex contraction of the n.m. is

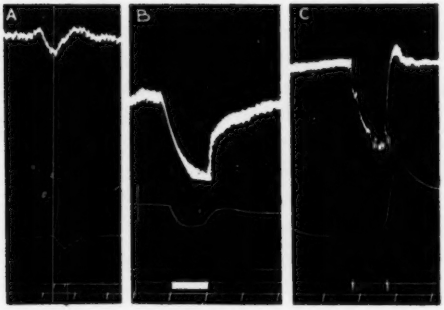


Fig. 1

Fig. 2

Fig. 1. Reflex relaxation of the nictating membrane. In this and the succeeding figures the cats were anesthetized with urethane and the adrenals tied off. The time (lowest tracing) is recorded in 30-second intervals.

A. Vagi cut. Carotids denervated. At signal right hamstring nerves stimulated centrally. Multivibrator, 4 volts, 12 per second.

B. Vagi cut. Carotids denervated. At signal left vagus stimulated centrally. Inductorium, coil distance: 7 cm.

C. Same animal as in B. At signal thorax compressed by hand.

Fig. 2. Carotids denervated. Vagi cut. At upper signal right sciatic stimulated centrally with condenser discharges; voltage: 10; capacity: 0.1μF; frequency: 2 per second. At the lower signals the left vagus was stimulated centrally with induction shocks, coil distance: 10 cm.

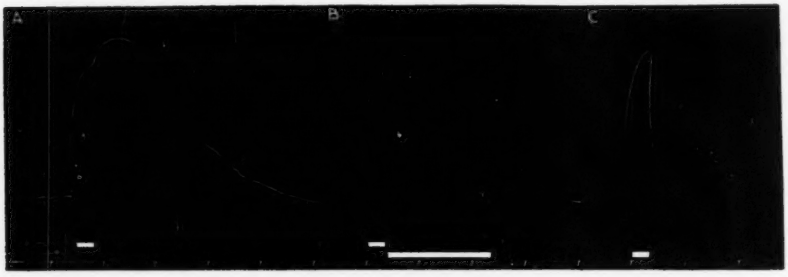


Fig. 3. Carotids denervated. Vagi cut.

A. Central stimulation of right sciatic. Inductorium, coil distance: 7 cm.

B. Same as A (upper signal) succeeded by central stimulation of left vagus, coil distance: 6 cm. (lower signal).

C. Peripheral stimulation of cut cervical sympathetic in the same animal. Coil distance: 11 cm.

usually attended by a rise of b.p. (figs. 4, 6 and 8D). If the suggestions just made were true, this rise of b.p. should set up depressor afferent impulses which would decrease the response of the n.m. Circulatory de-

afferentation should then lead to increased responses of the n.m. to a given stimulus. To test these ideas preliminary observations were made in animals in which the two vagi and the *right* carotid-sinus nerve were severed. Stimuli were applied to the sciatic and their effects were recorded with the *left* carotid artery open and closed. Figure 4 illustrates such an experiment and shows no significant differences between the responses obtained in the two conditions.

If the responses to sciatic stimulation are recorded, however, before and after section of both vagi and both carotid-sinus nerves, a striking increase of the reflex contractions obtains after the section (fig. 5).

E. *Rebound.* An excitatory rebound, i.e., a sudden increase of contraction at the end of stimulation, is frequently encountered (fig. 6). High

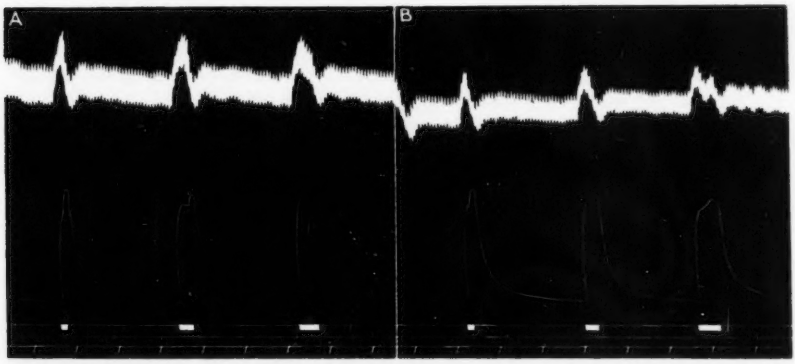


Fig. 4. Right carotid denervated. Vagi cut.

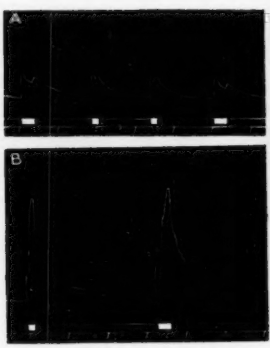
A. Central stimulation of the right sciatic (coil distance: 8 cm.) for 5, 10 and 15 seconds. The left common carotid was clamped throughout these observations.

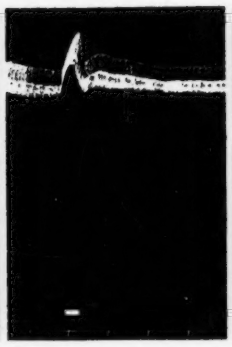
B. The clamp on the left carotid was released. Same stimuli as in A.

intensities of stimulation are especially favorable for this excitatory rebound. An inhibitory rebound (i.e., a relaxation below the tonic basal level after the initial contraction, which occurs during stimulation) is, on the contrary, rare and not marked. Figure 8B illustrates an instance.

F. *After-discharge.* Relaxation after direct stimulation of the cervical sympathetic is a rapid process, lasting as a rule less than 1 minute (fig. 3C). The subsidence of a reflex contraction is usually longer, and may attain a duration of several minutes (fig. 3A). This long subsidence implies a long after-discharge from the centers. A prolonged after-discharge is also illustrated by the responses in which an excitatory rebound occurs (fig. 6).

G. *Summation of reflex responses.* If two excitatory afferents are stimu-





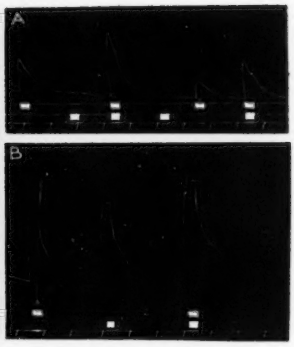


Fig. 5 Fig. 6 Fig. 7

Fig. 5. Responses to 5 and 10 seconds' stimulation of the right sciatic centrally (coil distance: 6 cm.).

A before and B after section of the vagi and depressors and carotid-sinus nerves.

Fig. 6. Right sciatic stimulated centrally (coil distance: 8 cm.).

Fig. 7. Carotids denervated. Vagi cut. The upper signal denotes central stimulation of the right sciatic and the lower of the left.

A. Coil distances: 13 cm.

B. Coil distances: 8 cm.

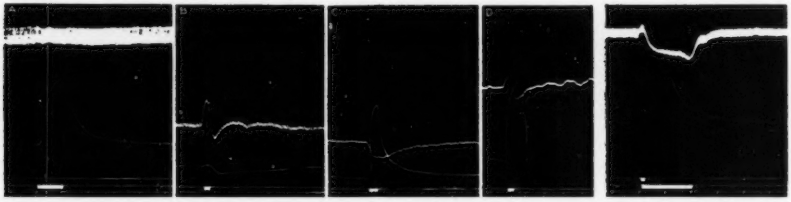


Fig. 8 Fig. 9

Fig. 8A. Central stimulation of the right sciatic. Multivibrator, 20 volts, 2 per second.

B. Central stimulation of the abdominal sympathetic chains at L5. Multivibrator, 4 volts, 20 per second.

C. Central stimulation of the hamstring nerves. Multivibrator, 26 volts, 10 per second.

D. Central stimulation of the right sciatic. Multivibrator, 8 volts, 20 per second.

Fig. 9. Carotids denervated. Vagi cut. Upper signal: right sciatic stimulated centrally (coil distance: 8 cm.). Lower signal: left vagus stimulated centrally (coil distance: 7 cm.).

lated first separately and then simultaneously, the response to the two stimulations is as a rule larger than that to either one alone. This summation is usually greater than lineal for minimal stimuli (facilitation) and

very scant for more intense and frequent stimuli (occlusion). Figure 7 illustrates typical instances.

H. *Lack of parallelism of the b.p. and n.m. responses.* All afferents thus far studied have effects on both the b.p. and the n.m. These effects are frequently parallel, i.e., contraction of the n.m. is associated with a rise of b.p. and relaxation with a fall (figs. 1A, 1B and 4).

The parallelism is, however, not invariably present. Figure 6 shows an instance in which it was absent. Furthermore, a definite change in either n.m. or b.p. can be obtained without any change in the other (fig. 8A and B), or they may respond in opposite directions (fig. 8C). The increased rises of b.p. in figure 4 after clamping of the left carotid, while the contractions of the n.m. were not modified, offer another instance of lack of parallelism. Still another interesting illustration of the independence of the two responses is seen in figure 8D, where an excitatory rebound in the n.m. was associated with an inhibitory rebound in the b.p.

This independence of the reflex effects was further tested by lowering of the b.p. while a reflex contraction of the n.m. was induced. Figure 9 records excitatory effects on the n.m. and a lowered b.p. produced by simultaneous stimulation of the sciatic and the vagus. Figure 1C shows that lowering the blood pressure by compressing the chest and opposing the venous return to the heart does not induce a relaxation of the n.m., but a contraction, probably due to asphyxia.

Discussion. All the features of the reflex responses of the n.m. described in the preceding sections are features likewise found in spinal reflexes (cf. Sherrington, 1906). The differences which may be met between autonomic and somatic spinal reflexes are quantitative, not qualitative, thus suggesting that the fundamental mechanisms underlying the reflex responses are probably identical for the two classes. The most striking quantitative difference discernible is in the time parameters, especially marked if we compare the intervals for effective central summation and the after-discharges. The source of this difference is yet unexplained. The fact remains that by multiplying the respective time scales by suitable factors all reflex responses thus far studied conform to a common pattern and this analogy of features is in favor of an analogy of processes.

One of us (Rosenblueth, 1934), impressed by the prolonged after-discharges occurring in cardiac reflexes, suggested that they are more readily explained, and with less subassumptions, if we postulate that the central excitatory state (c.e.s.) may attain supraliminal values (as could be the case if the central excitatory agent were a chemical substance—cf. Sherrington, 1925), than if we postulate only a liminal c.e.s. and a persistent bombardment of the neurones. In the present report we are again confronted by after-discharges which may last several minutes (figs. 3A, 6 and 8D). They are a direct consequence of the nerve impulses afferent

to the centers and not an indirect effect of other changes induced by the stimuli, such as alterations of blood pressure (section H, figs. 8 and 9). Our opinion is again that, although after-discharge might be due to a continuous bombardment by impulses traveling over self-reëxciting, reverberating paths (Ranson and Hinsey, 1930), it can be more simply explained in terms of a chemical mediation at the synapses. A firmer judgment must await direct evidence for either, or both, or alternative theories.

Rebound was obtained from all the excitatory afferents used (section A). This leads to the inference that all these nerves are mixed, containing both excitatory and inhibitory afferents (see Sherrington and Sowton, 1911). The mixed nature of the nerves makes uncertain the interpretation of the phenomena of summation (section G). For example, the rôle which inhibition might play in a response showing apparent occlusion (fig. 7B) can not be determined without further experimental analysis.

Information is inadequate with regard to the receptors from which the stimulated afferent nerves arise. Accordingly, little may be said concerning the physiological significance in the organism of the reflexes which we have studied.

An interesting property common to these and other autonomic reflexes is the frequent lack of "local sign." Some vasomotor reflexes bear a local sign, e.g., the Lovén and the axon reflexes; many others, however, both pressor and depressor, do not. The effects are then generalized, and no specific changes are noticeable in the territory where the afferent originates. Cardiac reflexes do not bear a local sign, nor do the n.m. reflexes here described.

Reflex contraction of the n.m. coincides as a rule with acceleration of the heart, rise of b.p., secretion of adrenine, dilatation of the pupil, salivation, etc. In all these responses an increased sympathetic discharge is or may be involved. On the other hand, erection of the hairs has not been noted; and it is possible to separate some of the responses (section H). It appears, therefore, that the effects are not obtained from stimulation of a main sympathetic center but from independent centers concerned with the different functions mentioned. A more detailed study is necessary, which should include a comparison of various anesthetics; thus cardiac and vasomotor reflexes are readily obtained under chloralose, but reflex contraction of the n.m. is absent. The principle of generalized sympathetic activity (Cannon, 1930) is, however, at present the only way of accounting for contraction of the n.m. on stimulation of a saphenous or a hypogastric nerve.

SUMMARY

Reflex contractions and relaxations of the smooth muscle in the nictitating membrane of the cat are described. The vagus is a suitable afferent to

demonstrate reflex relaxation (figs. 1, 2 and 3). Section of the vagi and denervation of the carotids increase the contractions elicited from the sciatic (fig. 5). Excitatory and inhibitory rebounds may be recorded (figs. 6 and 8B). After-discharge may be very prolonged (figs. 3A, 6 and 8D). Simultaneous stimulation of two afferents leads to summation of the reflex effects (fig. 7). The responses of the nictitating membrane are direct and not secondary to changes of blood pressure (figs. 1, 4, 6 and 8).

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THE EXTRAHEPATIC BILIARY TRACT DURING ANAPHYLAXIS

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This report covers a study of the gall bladder during anaphylaxis following protein sensitization. It comprises three parts: 1, the reaction of the empty gall bladder to the antigen when the vesicle was suspended as a strip in a physiologic solution; 2, the pressure induced within the gall bladder when antigen was added to the bath in which the vesicle was suspended; and 3, the reaction of the sphincteric mechanism, at the duodenal end of the common bile duct, to the antigen when it was added to the bath.

Ivy (1) has recently made a most excellent review of the literature on the physiology of the gall bladder, and there appears to be but little experimental work directly bearing on this phase of physiology of the gall bladder during anaphylactic shock.

METHODS. Young male guinea pigs that had been previously sensitized by the subcutaneous injection of 0.1 cc. of horse serum and 0.1 cc. of egg white (chicken) were used. The guinea pigs were ready for use about three weeks after the injection.

The animals were killed by a blow on the head, the abdomen was quickly opened, and the extrahepatic biliary tract, together with the portion of duodenum containing the sphincter of the common bile duct, was removed and placed in Tyrode's solution. At the same time, a small segment of intestine and of the urinary bladder were removed, and in some experiments these were tested for the anaphylactic reaction, along with the gall bladder. In part 1 the gall bladder was severed from the rest of the biliary tract, all bile was expressed, and then one end was tied to an arm of a small glass frame. The strip of intestine and the strip of urinary bladder were likewise tied to adjacent arms of this glass frame, and then all were suspended in a bath of 50 cc. of Tyrode's solution, which was maintained at a constant temperature of 38.5°C. The other ends of the three structures were each tied to a lever in a manner usually employed for recording the movements of intestinal strips. In part 2 the gall bladder was incannulated, perfused a number of times to free it of all bile, and then was immersed in the bath. By means of a small rubber tubing, one arm of a

T-tube connected the incannulated gall bladder with a buret, while the other arm led to a water manometer that was fixed along the front elevation of the photographic kymograph that was used to record the changes which were observed. The buret served as a convenient means of filling, or removing fluid from, the gall bladder. The strip of intestine was fixed to the glass frame, as in part 1, immersed in the bath along with the gall bladder, and tied to a recording lever, the end of which moved freely in front of the manometer. Thus, the movements of the water column in the manometer and the movements of the lever were recorded photographically at the same time. In part 3 the extrahepatic biliary tract was removed in continuity with that portion of the duodenum that contained the sphincter of the common duct. The hepatic duct, just above its junction with the cystic duct was incannulated and all other biliary tributaries were ligated. After washing the biliary tract free of all bile, this preparation was immersed in a bath of 50 cc. of Tyrode's solution and connected by means of the T-tube to the buret and to the manometer as described previously. A strip of intestine was likewise attached to the recording lever. The biliary tract preparation was so arranged, when suspended in the bath, that the neck of the gall bladder and the duodenal segment, including the sphincter, were at the same level. We employed our photographic recording device (2) to record all changes that were induced in these organs during the reaction to the antigens.

In order to test for the anaphylactic response in these preparations, the antigen, either horse serum or egg white, was added directly to the 50 cc. physiologic bath in which the tissues were suspended. The amount of the antigen which was added was always first diluted by adding enough of the bath solution to make 1 cc., and then was added to the remaining 49 cc., so that the concentration of the specified antigen was the actual concentration in 50 cc. of the solution.

EXPERIMENTAL OBSERVATIONS. *1. The reaction of the empty sensitized gall bladder to the antigen.* In order to secure adequate oxygenation and a normal muscle tonus, the gall bladder, urinary bladder, and intestinal strip were allowed to remain in the Tyrode's solution for fully fifteen minutes before testing for the anaphylactic response. When horse serum, in a dilution of 1 to 2500, was added to the bath solution in which these organs were immersed, there was a marked contraction (fig. 1, *no. 1*). The response of the intestine was more immediate than that of the urinary bladder or of the gall bladder, and in all three there was a brief latent period before the reaction to the antigen was manifested.

After changing the solution twice and after a brief rest period, during which the organs regained their normal tone, egg white was added to the bath (fig. 1, *no. 2*). A concentration of 1 to 5000 of egg white produced a comparable, but more marked, contraction of all three organs.

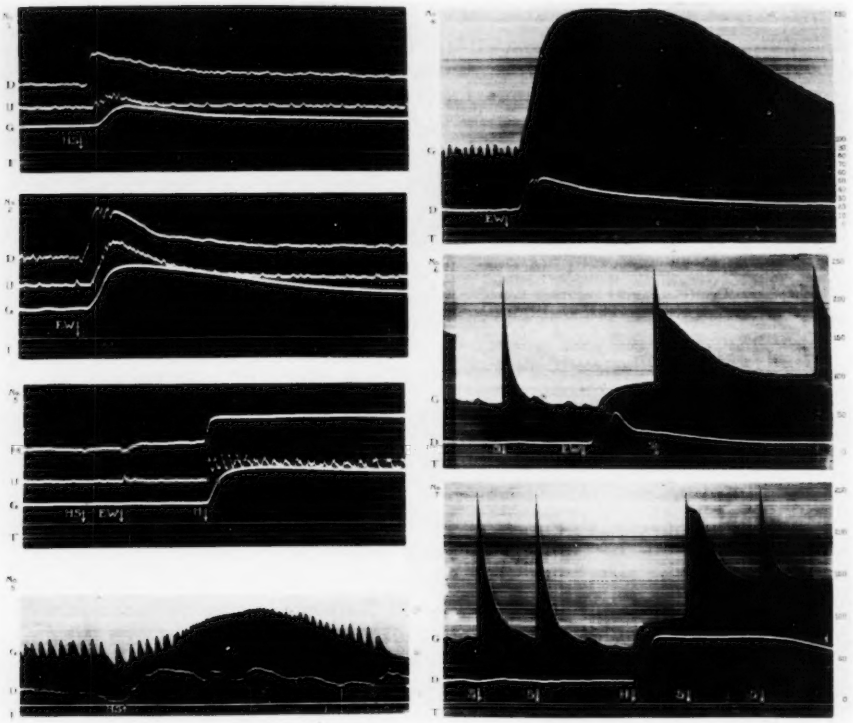

Fig. 1. In all tracings *D* represents duodenum; *U*, urinary bladder; *G*, gall bladder; *T*, time (between long dashes equals 1 minute).

Nos. 1 and 2. Same preparation in both. Effect of adding different substances to the bath: *HS*, horse serum, 1 to 2500; *EW*, egg white, 1 to 5000.

No. 3. Same preparation as that in *nos. 1 and 2.* It is evident that the preparation had been desensitized to horse serum and to egg white by the procedures represented in *nos. 1 and 2.* Addition to the bath of histamine, *H*, 1 to 100,000, demonstrated viability of the preparation.

Nos. 4 and 5. Pressure within the gall bladder, measured in millimeters of water. In *no. 4*, *EW* represents egg white, as before. The animal represented in *no. 5* had been weakly sensitized to horse serum. *HS*, horse serum added to bath.

Nos. 6 and 7. Reaction of sphincter of common bile duct. Records are in millimeters of water. *No. 6*, *s* represents test of resistance offered by sphincter before inducing anaphylaxis by adding the antigen, egg white, *EW*, to the bath; *s'*, after anaphylaxis. *No. 7.* Reaction of sphincter before, *s*, during, and after *s'*, addition of histamine, *H*, 1 to 100,000 to the bath.

In order to determine whether this was a true anaphylactic response, we changed the solution in the bath three times, adding fresh Tyrode's solution each time, and then added a concentration of the horse serum three times as great as that which was added before. This time there was no contraction of either the gall bladder or the urinary bladder and only a slight response by the intestinal strip (fig. 1, *no. 3*). Likewise, when a concentration of egg white that was three times as great as that which was used the first time was added to the bath, the gall bladder did not contract, although there was a slight response by the urinary bladder and by the intestinal strip. Thus, desensitization had occurred and the responses, which are recorded in figure 1, *nos. 1 and 2*, and which followed the addition of the antigens, were typical anaphylactic responses. In order to test the viability of these organs, we added histamine to the bath, in a concentration of 1 to 100,000 (fig. 1, *no. 3*). There was an immediate response and all organs contracted without any intervening latent period. This immediate reaction was typical of all histamine reactions and different from the anaphylactic changes which follow after a brief latent period.

2. Intra-gall bladder pressure during anaphylaxis. Although our first experiments had demonstrated clearly an anaphylactic response by the gall bladder to either horse serum or egg white, we wished to observe the changes in pressure that occurred within the vesicle during these responses, and to compare them with the pressure which occurs in a normally contracting vesicle.

By means of the buret and the T-tube connection with the gall bladder and the manometer, as described previously, the biliary vesicle was slowly filled with the physiologic solution until such pressures, at which normal gall bladder rhythm was induced, were reached. On the addition of 0.01 cc. of egg white to the 50 cc. Tyrode bath, making a concentration of 1 to 5000, there was a marked contraction of the gall bladder and the intestinal strip. The gall bladder pressure rose rapidly from 75 mm. to 250 mm. of water. This high pressure level was maintained for three minutes, when a gradual relaxation occurred and the pressure was gradually reduced (fig. 1, *no. 4*). We encountered this rather powerful contraction of the gall bladder, which raised the pressure to 250 mm. of water, only once, but usually during such an anaphylactic response the pressures increased from 60 or 70 mm. to 160 or 200 mm. of water. These pressures, during anaphylaxis, were invariably higher than those which a contracting gall bladder of a guinea pig usually exerts and they probably represent the maximal pressure against which the vesicle may contract.

After the preparation had been washed, we tested for the anaphylactic response a second time, using a triple concentration of the same antigen. As there was no response or contraction by the gall bladder, it was certain that desensitization had occurred, and that the contraction we had ob-

served was definitely the result of anaphylaxis. When we added histamine to the bath solution, in a concentration of 1 to 100,000, the gall bladder contracted immediately, thus demonstrating the viability of the preparation.

When animals were weakly sensitized to either protein, or when submaximal doses of antigen were added to the bath containing the isolated gall bladder, only slight contractions of the viscus were observed. In the experiment (fig. 1, *no. 5*), the animal had been weakly sensitized to horse serum. On the addition of the antigen, in dilute concentration to the bath in which the gall bladder was suspended, there was an immediate and sustained reaction by the duodenum but a more gradual response by the gall bladder. This retarded reaction of the gall bladder showed clearly the relation of rhythm to tonic contraction. During the two-minute interval after the addition of the antigen to the bath, there was a slight increase in the tonic contraction of the vesicle as evidenced by an increase in pressure without any appreciable change in either the frequency or the amplitude of rhythm (fig. 1, *no. 5*). As tonic contraction increased and as the intravesical pressure rose from 40 to 100 mm. of water, the amplitude of rhythm gradually decreased. The frequency of rhythm, however, increased from 3 or 4 cycles per minute to about 8 or 10 per minute. After the full force of the contraction had passed and a gradual relaxation was in progress, even without washing, a rhythm of normal amplitude and frequency returned (fig. 1, *no. 5*). Rhythm was dependent on an optimal pressure within the gall bladder and will not occur at intravesical pressures either too low or too high.

3. The reaction of the sphincter of the common bile duct during anaphylaxis. When animals were observed during an anaphylactic response, we found that the gall bladder was usually well distended with bile. Since the vesicle, in vitro, did contract, often with exceedingly high pressures, in the presence of antigen, it was of interest to know whether the sphincteric mechanism at the duodenal end of the common bile duct likewise contracted and perhaps inhibited the evacuation of the vesicle in the intact animal. We wished to know whether or not the gall bladder pressure during an anaphylactic response was greater or less than that which is exerted by the regulatory sphincter at the duodenal end of the common bile duct.

The extrahepatic biliary tract, together with a segment of duodenum which contained the common duct sphincter, was suspended in the Tyrode bath in the manner described previously. After a brief rest interval to allow for thorough oxygenation, the gall bladder was slowly filled through the cannula attached to the buret. The pressure in the preparation was slowly increased and, when pressures of 70 or 80 mm. of water were recorded on the manometer, rhythmic contractions were evident in the gall bladder. The sphincteric mechanism withstood these pressures and fluid

did not pass through the ampulla into the bath; but if more fluid was added from the buret, so as to increase greatly the pressure, all rhythm in the gall bladder then ceased and the resistance of the sphincteric mechanism was overcome, thus permitting the escape of the fluid.

The resistance of the sphincteric mechanisms was tested in the following manner: the physiologic solution was permitted to flow from the buret into the gall bladder preparation until a pressure of 230 mm. of water was recorded on the manometer. With such a pressure the gall bladder was overdistended and the resistance of the sphincter was overcome, so that fluid immediately passed from the preparation through the ampulla and the pressure immediately decreased (see first part of fig. 1, *no. 6*). When pressure levels, which the sphincter normally withstood, were reached, the ampulla closed, no further fluid escaped into the bath, and rhythmic contractions were resumed by the gall bladder (fig. 1, *no. 6*). The pressure exerted by the sphincteric mechanism was quite constant and hovered around the level at which normal rhythm occurred.

Having learned what the normal resistance offered by the sphincter for any such preparation was, we were prepared to test the reaction of the sphincteric mechanism during anaphylaxis. When egg white, in a concentration of 1 to 1000, was added to the bath, there was a brief latent period, which was followed by a typical response in both duodenal and hepatic duct preparations (fig. 1, *no. 6*). The immediate increase in pressure from 60 to 93 mm. represents the maximal contraction of the gall bladder during its response to the antigen. After about two minutes, the pressure in the system was raised, by adding fluid from the buret in the manner previously indicated, to about 250 mm. The effect, which was induced on the sphincter by the antigen, was clearly demonstrated by the contour of the curve which was described by the water level as it much more slowly descended in the manometer (fig. 1, *no. 6*). Whereas it required but one minute, in the test prior to adding antigen, for the fluid in the manometric system to pass the ampulla and to come to rest at the usual pressure level, it required about five minutes after the antigen had induced a contraction of the sphincter. But what is more significant is that the pressure, which was withstood by the sphincteric mechanism during the anaphylactic response, was 40 mm. more than before, and was greater by 10 mm. than the maximal pressure which was exerted by the gall bladder. This result occurred in all experiments undertaken and showed definitely that during an anaphylactic response the resistance which was offered by the sphincter at the duodenal end of the common bile duct was greater than the emptying power of the gall bladder. Thus it may be that during anaphylaxis the gall bladder does contract but may not empty because of the inhibiting power that is exerted by the sphincter at the end of the common bile duct.

When we added histamine, in a concentration of 1 to 100,000, to a bath which contained such a preparation of the extrahepatic biliary tract that had been washed repeatedly, a similar result was attained (fig. 1, *no 7*). In this case, there was no latent period; the strip of duodenum responded in the usual way to histamine and the pressure within the gall bladder rose from 65 to 100 mm. of water. When we tested the sphincteric resistance by again filling the manometric system to 250 mm. and permitting fluid to flow through the ampulla, we learned that the resistance during histamine response had increased about 70 mm. and that the sphincteric mechanism now withstood a pressure of 140 mm. of water. The pressure which was exerted by the sphincter during histamine response exceeded that which was exerted by the gall bladder by about 40 mm. of water. Thus, in the histamine reaction, as in anaphylaxis, the resistance that is offered by the sphincter at the duodenal end of the common bile duct is greater than the force that is exerted by the contraction of the gall bladder, and probably is of significance in explaining why the gall bladder fails to empty.

SUMMARY

This report covers a series of observations on the isolated extrahepatic biliary tract of guinea pigs, during anaphylactic responses to foreign proteins to which the animals had been previously sensitized.

All animals were sensitized by the subcutaneous injection of 0.1 cc. of horse serum and 0.1 cc. of (chicken) egg white. Three weeks later, they were killed and the extrahepatic biliary tract was removed, placed in Tyrode's solution, and subsequently tested for the anaphylactic response.

The observations reported in this paper warrant the conclusions that the biliary tract, including the gall bladder, may become sensitized to foreign proteins equally as well as any other organ, and that it will contract vigorously in the presence of the antigen.

When antigen in a concentration of 1 to 2500, or 1 to 5000, was added to the bath of Tyrode's solution in which the gall bladder was suspended, contraction of the vesicle followed after a brief latent period. This was true of a strip of duodenum and a strip of the urinary bladder, which likewise were suspended in the bath with the gall bladder.

When the antigen was added to the bath in which a rhythmically contracting gall bladder was suspended, the intravesical pressure immediately rose from levels of 60 or 70 mm. of water to levels as high as 200 or 240 mm. of water. These are pressures which are higher than those usually encountered in a normally contracting gall bladder.

When antigen was added to the bath in which the entire extrahepatic biliary tract, together with the sphincter at the duodenal end of the common bile duct, was suspended, there was a contraction by both the gall bladder and the duodenal sphincter. The force or pressure which was

exerted by the sphincteric mechanism was found to be greater than that which was exerted by the gall bladder. This may explain the observations hitherto made, that the gall bladder ordinarily does not empty during an anaphylactic shock. The gall bladder does contract, but the inhibitory influence of the sphincteric mechanism prevents the flow of bile into the duodenum.

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THE EFFECT OF INTRAVENOUS INJECTIONS OF AMINO ACIDS ON THE MOTILITY OF THE STOMACH IN NORMAL AND FASTING DOGS

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There have been many theories expressed concerning the cause of hunger contractions of the empty stomach. The relationship of the level of blood sugar to the hunger activity has been investigated by many workers. There is not a unanimity of opinions among them. A bibliography will be found in the papers of Mulinos (1933), and LaBarre (1931). It is not clear to us just what is the cause, or causes, of the periodic gastric motor activity seen in hunger.

Being unaware of any studies on the effect of amino acids upon the hunger contractions, it seemed possible that data along that line might to some degree throw light upon the question.

METHOD OF STUDY. Three healthy dogs, having gastric fistulae, and weighing between 12 and 14 kgm., were used. They were thoroughly trained to lie quietly on a table while motility tracings were being made by the balloon method. The same general procedure and equipment were used in these experiments as have been used in various papers reported from this laboratory in recent years.

The animals were allowed to go without food for a period of eighteen to twenty-four hours before each experiment. Normal controls were run on each animal for several days before injections of amino acids were made. Injections of saline were used to rule out the effect of the injection per se.

Various amino acids were prepared by one of us (E. U. S.), and recrystallized until the constants closely approximate the values accepted for the pure substances. The amino acids were dissolved in a small volume of neutral saline. Injections were made at various times with respect to the state of gastric motility, i.e., at the beginning of a quiescent period, at the beginning of activity, at the height of activity or at the end of activity. Table 1 shows the results of this study. This table was constructed so as to show how long the particular activity of the stomach had been going on at the time the injection was made; and how long after the injection before there was noticeable change.

The possibility was suggested that not a single amino acid was involved

TABLE 1

The effect of intravenous injection of amino acids on the motility of the empty stomach

AMINO ACID	AMOUNT	DOG NO.	LENGTH OF PERIOD PRECEDING INJECTION	MINUTES AFTER INJECTION BEFORE CHANGE IN MOTILITY
	mgm.		*minutes*	*minutes*
Glycine	100	1	57	125
	100	2	18	35
Cystine	21	1	30	60
	20	1	8	8
	30	1	9	11
	21	2	18	33
	20	2	32	110
	30	2	0	8
Leucine	50	1	5	13
	50	2	20	31
Isoleucine	50	1	30	40
	50	2	18	52
Tyrosine	25	1	15	51
	35	1	4	7
	25	2	56	53
	35	2	?	?
Proline	50	1	120	10
	50	2	131	40
Tryptophane	26	1	49	81
	26	2	10	45
Lysine	50	1	16	53
	50	2	15	56
Arginine	15	1	11	4
	35	1	17	12
	15	2	33	13
	35	2	6	83
Histidine	15	1	85	50
	21	1	90	21
	18	1	111	18
	15	2	92	43
	21	2	15	12
	18	2	24	29

TABLE 1—*Concluded*

AMINO ACID	AMOUNT	DOG NO.	LENGTH OF PERIOD PRECEDING INJECTION	MINUTES AFTER INJECTION BEFORE CHANGE IN MOTILITY
	mgm.		*minutes*	*minutes*
Glutathione	11	1		60
	15	1	34	35
	10	1		
	8	2	18	12
	15	2	32	30
	10	2	32	57
Creatine	50	1	18	17
	50	2		

but rather a group of amino acids. Therefore, we planned experiments based upon the assumption that hunger was abolished upon the absorption of the amino acids contained in a protein digest. We prepared a mixture of amino acids in the proportion found in casein. Solutions containing 600 mgm. of the mixture were injected intravenously under the conditions described above. There was no apparent effect upon the hunger motility of the stomach.

Discussion. So far as we know, there are little data available on the concentration of various amino acids in the blood. Abderhalden (in 1913) showed, however, that proline, leucine, valine, alanine, glycine, aspartic acid, glutamic acid, tryptophane, lysine, arginine and histidine were present. The average figure for the non-protein amino nitrogen in the blood of the fasting dog is 4.4 mgm. per 100 cc. of blood. This indicates that about 31 mgm. of amino acids are found in 100 cc. of the blood. Assuming our dogs have a blood volume of 1 liter, the total circulating amino acids amount to 300 mgm. (fasting). Since at the height of absorption the amino acid content of the blood is about 100 per cent above the fasting level, the increase in our animals is estimated to be about 300 mgm.

In one group of experiments we have injected the individual amino acids in such amounts as would increase their particular concentration by 100 per cent or more. In another group of experiments we have injected a mixture of amino acids (approximating the composition of casein), in such amounts as would increase the total amino acid concentration of the blood by 200 to 300 per cent. Neither group of experiments indicated that the injections altered the state of gastric hunger motility, neither in the direction of augmentation nor inhibition. This was true when the injections were made during any phase or state of activity and, therefore, the state of the stomach was not considered to be a complicating factor.

CONCLUSIONS

Inasmuch as our experiments (43 in number) on three dogs have not provided evidence indicating that the length, amplitude or frequency of periods of gastric motility are modified by the intravenous injection of purified amino acids, we are of the opinion that the level of amino acids in the blood is not a contributing factor to the hunger contraction mechanism.

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THE DISAPPEARANCE OF DIGESTIVE INHIBITION WITH THE REPETITION OF EXERCISE[1]

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In our first experience with the effects of exercise upon the acidity of the gastric juice we noted that the depressing influence of muscular work progressively lessened with repetition of the effort (Hellebrandt and Miles, 1932). This was assumed to be a training effect. Subsequently the same observation was made under circumstances which definitely precluded calling it training in the usual sense of that term, which implies the development of adaptations facilitating the performance of work as a result of systematic, long repeated exercise. Inhibition vanished after a few trials of the prescribed work had been superimposed at long and irregular intervals upon the strenuous activity program of one inured to much exercise (Hellebrandt and Hoopes, 1934). Steinhaus (1931) had stressed the importance of emotional factors. There was reason to think that exercise without feeling does not have the same effect on gastric function as that associated with psychic stress. The object of this study was therefore, to emancipate exercise from its psychic concomitants in man; to observe the effects of work alone upon gastric secretory function; and finally to introduce the emotional element, either by itself or in conjunction with exercise.

METHODS AND RESULTS. The subjects were healthy young adult women, students or graduates of a professional course in physical education, habituated to vigorous exercise, familiar with the technique of gastric intubation and physiologic experimentation. There were 6 subjects upon whom 75 fractional analyses were made under resting conditions. Secretion was stimulated by 50 cc. of 7 per cent alcohol or by histamine. The methods of aspiration and titration were the same as in our previous work. The alcohol was warmed and introduced through the stomach tube. Samples of the gastric contents were removed at quarter hour intervals. Histamine was given hypodermically in a dosage of 0.01 mgm./kgm., using

[1] This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

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a 1:1000 solution of ergamine acid phosphate. The total secretion was aspirated every 15 minutes. Laboratory exercise was performed on 74 days, on 34 of which the gastric response was also studied. There were in addition 34 single aspirations, 6 under resting conditions and 28 associated with exercise. Observations were likewise made when natural situations accompanied by emotional disturbances were experienced. A few attempts were made to simulate psychically agitating occasions.

1. Fractional analysis after alcohol. It is difficult to find a measurable generalized exercise so monotonous and readily stereotyped as pedalling the stationary bicycle at a constant speed against a fixed load. Working for 15 minutes at 740 kgm. m./min. with an average pedalling rate of 73 R.P.M. approached the limits of endurance of our subjects. This work was performed on consecutive days by two subjects. Graph I, figure 1, illustrates the response of S. L. H. and shows by the sixteenth day the depression had disappeared. *Very severe exercise can be performed without inhibiting the acid secreting mechanism of the stomach.* Subject S. L. H. continued exercising daily, either alone or in the presence of technical assistants. On the 39th day she performed in public demonstration before a mixed audience. The experience was without effect upon the acidity response as shown in graph II, figure 1. *An emotionally upsetting situation may be met without suppressing gastric function.*

The repetition of work is associated with the acquisition of a trained state. Seasoned to meet the demands of a given physical task, the subject is only partially trained for more severe grades of effort (Steinhaus, 1933). If the eventual ability to carry on gastric work undisturbed during and after exercise is a part of the complex training phenomenon, increasing the difficulty of the muscular exertion should be accompanied by a reversion to the state of secretory inhibition. On the 44th consecutive day of exercise the subject preceded the usual 15 minutes of severe work by an exhaustive bout of exercise lasting for one hour. Graph III, figure 1, shows that this had almost no effect on the way in which her stomach secreted HCl in response to the standard test meal of alcohol. On the 46th day the severity of the routine effort was pushed to the limit of the subject's physiological ability, 1031 kgm. m./min. at a pedalling rate of 88 R.P.M. Circulatory and respiratory distress was acute. At the cessation of work there was perioral blanching, very labored breathing and threatened collapse. Graph IV, figure 1, shows that in spite of exercise violent to the point of prostration gastric function proceeded unimpaired. This is striking evidence that the mere contraction of muscle and the vasomotor shifts associated with generalized exercise are in themselves powerless to suppress this phase of gastric function.

Subsequently S. L. H. re-trained for the same exercise to note if freedom from suppression would come on more promptly. By the ninth day all

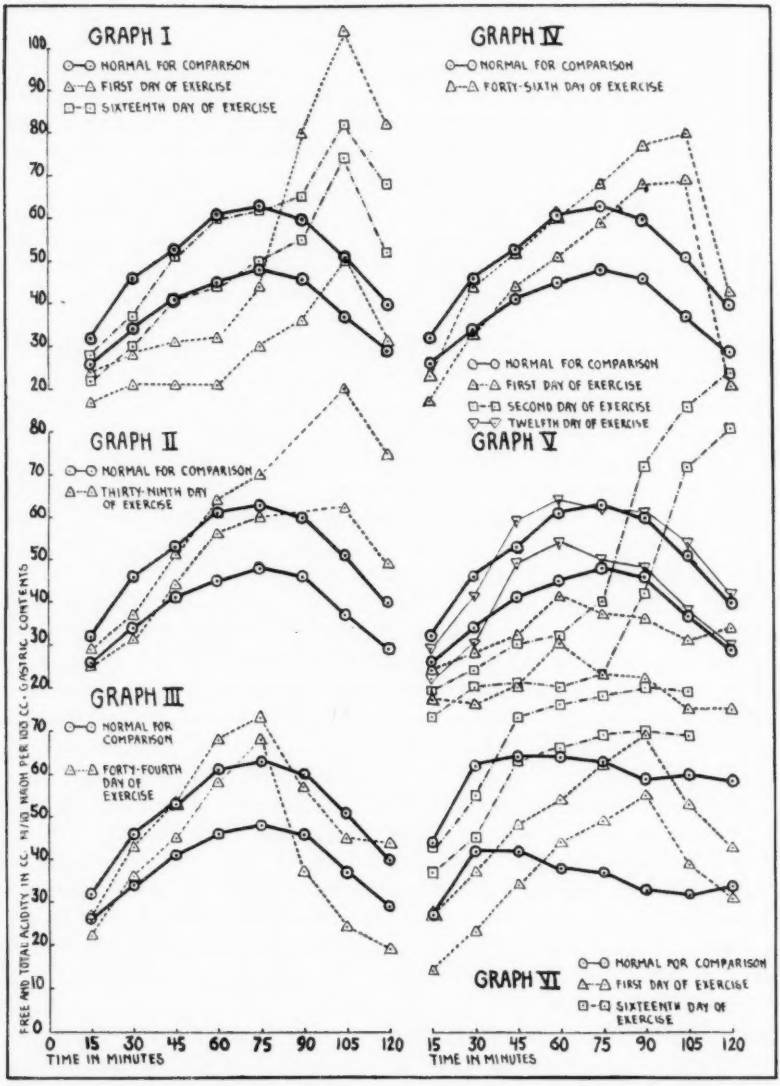

Fig. 1. Graphs showing the acidity response to a test meal of alcohol under the following conditions: I. Repetition of work by subject S.L.H. II. Psychic disturbance superimposed upon repeated work. III. and IV. Increasing the severity of the work. V. Confirmatory series of S.L.H. on repeated work. VI. Repetition of work by subject F.A.H.

evidence of the characteristic first hour inhibition had disappeared and by the twelfth the titration curve was approximately the same as that at rest (graph V, fig. 1). Graph VI, figure 1, demonstrates the main results of a similar series of observations on another subject. The exercise could not be carried out by F. A. H. without cardiac strain. Examination on the 16th day indicated enlargement to percussion and orthodiagraphic study confirmed the impression. It seems significant that work exhaustive enough to bring on cardiac enlargement should be associated with so little change in gastric function.

2. Fractional analysis after histamine. The administration of histamine was always accompanied by brilliant flushing, a sensation of heat, and transient throbbing headache. Unpleasant symptoms were notably alleviated by exercise. The second time work was performed immediately after the injection of histamine, the first post-exercise gastric sample was slightly discolored. The histamine juice at rest had been clear, colorless and limpid. As the experiment was repeated coloration increased until on the fourth trial all post-exercise samples were tinged to the hue of highly colored urine. Many contained a chocolate brown flocculent precipitate. All samples were positive for blood.

Histamine produces capillary dilatation (Krogh, 1930). That the permeability of the vessel wall is decreased is evidenced by the edematous induration at the site of the injection. The high systemic blood pressure of severe exercise may, under such conditions of capillary paralysis, be responsible for the appearance of blood in the stomach secretions. Bleeding was not due to trauma by the tube. We have examined more than 1200 gastric samples for occult blood and have ample evidence that in our tube-trained subjects the aspiration of the fasting residuum and the gastric contents after stimulation by gruel, alcohol or histamine may be accomplished without occult or visible bleeding. This is true also of post-exercise gastric aspiration when alcohol is the secretory stimulant. It may be of practical importance to know whether histamine is contraindicated in the presence of vascular hypertension or whether repetitious administration is harmful. Fluoroscopic and radiographic examination showed no gastric abnormality in the two subjects upon whom the observation was first made. At no time in the limited series of experiments completed did exercise significantly suppress the secretory response to this powerful stimulant. The exercise curves were like those obtained at rest as illustrated in graph I, figure 2.

3. Single aspirations. A one hour test breakfast of 300 cc. of warm water and 4 arrowroot cookies was administered in the usual way. Subject J. H. performed the severe 15 minute exercise immediately after its ingestion. At first distinctly depressing, the one hour acidity gradually returned toward and eventually superseded normal (graph II, fig. 2). The

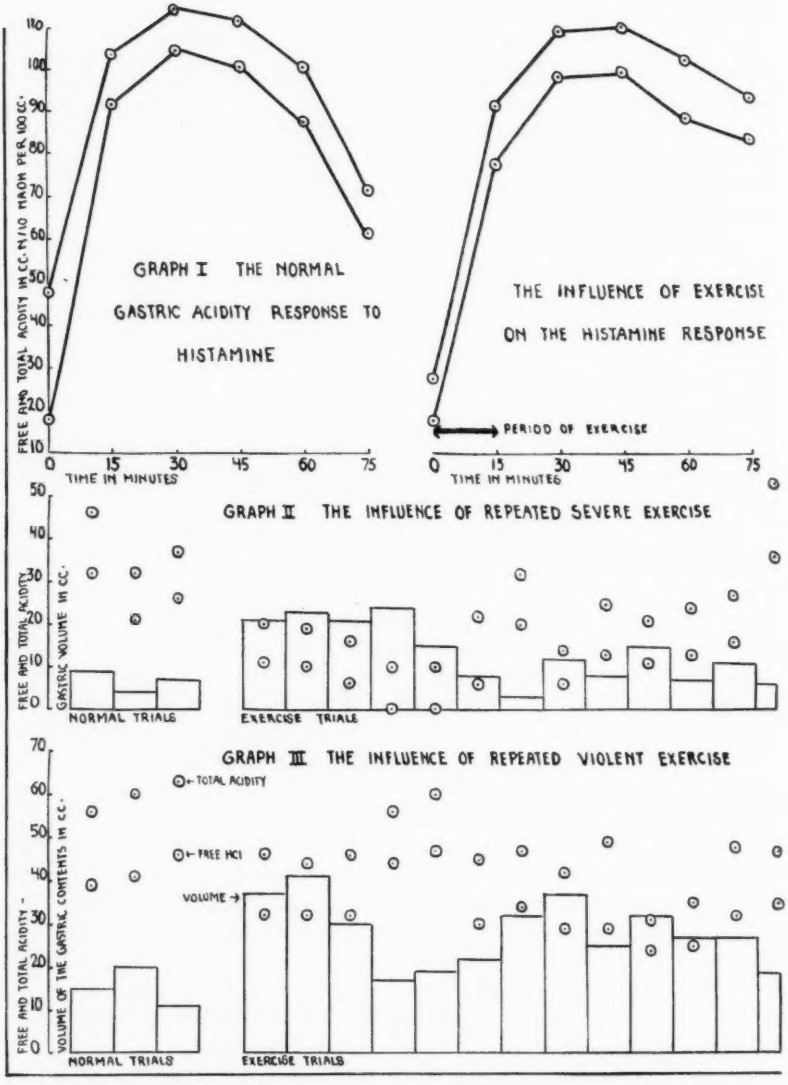
FREE AND TOTAL ACIDITY IN CC. N/10 NAOH PER 100 CC.
GRAPH I THE NORMAL GASTRIC ACIDITY RESPONSE TO HISTAMINE
TIME IN MINUTES
THE INFLUENCE OF EXERCISE ON THE HISTAMINE RESPONSE
PERIOD OF EXERCISE
TIME IN MINUTES
GRAPH II THE INFLUENCE OF REPEATED SEVERE EXERCISE
FREE AND TOTAL ACIDITY
GASTRIC VOLUME IN CC.
NORMAL TRIALS
EXERCISE TRIALS
GRAPH III THE INFLUENCE OF REPEATED VIOLENT EXERCISE
TOTAL ACIDITY
FREE HCl
VOLUME
FREE AND TOTAL ACIDITY -
VOLUME OF THE GASTRIC CONTENTS IN CC.
NORMAL TRIALS
EXERCISE TRIALS

Fig. 2. Graph I. The influence of exercise upon the gastric acidity response to histamine. Graphs II and III. The influence of exercise, severe and violent respectively, upon the volume and acidity of the gastric contents aspirated one hour after a test meal of water and arrowroot cookies.

large volumes of the 60 minute aspirations on the first few days of exercise indicate motor inhibition, which in itself contributes by dilution to the low acidity values. R. E. B. studied the effect of a short bout of violent work at high speed, 1031 kgm. m./min. for 120 seconds and 740 kgm. m./min. for 60 seconds at pedalling rates of 88 and 73 R.P.M. respectively. The exercise was as acute as the subject could tolerate. Except for approximately normal findings on the fourth and fifth days, the acidities remained persistently low and were accompanied by abnormally high gastric volumes (graph III, fig. 2). The subject never performed the required work without distress. Relating the rigor of the exercise to the ability of the subject to perform it, this represents the hardest work done in our series. Even under this stringent regime, although suppressed, the stomach showed remarkably good ability to continue its work.

4. Natural experiences associated with emotional stress. Excitement, anticipation of unusual events, emotional disturbances and the like derange gastro-intestinal function. Cannon (1909, 1929) has collected much evidence in support of this and the arresting effects of psychic disturbance have been demonstrated under hypnosis (Bennet and Venables, 1920; Luckhardt and Johnston, 1924). McDowall (1927) considers even minor stress of sufficient importance to digestion to make its control and prevention a practical problem in industrial hygiene. However, we observed that in *tube-conditioned subjects*, emotional stress severe enough to induce dryness of the mouth and palpitation, left the acid secreting power of the stomach essentially unaltered. Any athlete who has participated in a tournament knows that the anticipatory excitement is often great even in experienced players. Our efforts to obtain objective evidence of this in terms of a modification of the gastric acidity response to a constant stimulus were also uniformly unsuccessful.

Discussion. The findings suggest that the disappearance of the inhibition of gastric secretory activity which commonly accompanies severe exercise when that muscular activity is repeated is not a training effect in the usual conception of that term. It appears too early to be so classified and once overcome, it is impossible again to induce it even with extreme grades of the exercise to which the subject has become familiarized. Professor Ragsdale of the Psychology Department of this University suggested that the phenomenon might be explained on a basis of "visceral learning." The studies of Coghill (1929) relating behavior to the anatomical development of the nervous system give evidence for the belief that motor skill appears not by grouping simple paths into a complex organization, but by emancipating the appropriate reaction from the initial diffuse response (Coghill, 1930; Herrick, 1931). Individuation may be explained in terms of Fulton's concept of the myotatic reflex (1926). It is every day experience that the initial reaction to a strange situation calls forth a widespread

response and a generalized type of behavior. Just as afferent impulses from key muscles serve to regulate the pyramidal discharge, so the viscera probably report that the sympathico-adrenal mechanism need not be called into play. In the athletic history of our subjects we find a plausible explanation of the rapidity with which the exercise suppression of gastric function disappears. They have been trained to acquire automatized behavior patterns in the realm of neuromuscular skills. They quickly emancipate reactions appropriate to the situation from the initial diffuse response. That the viscera share in this efficiency of regulation is probable, but in the domain of speculation.

The technique of gastric intubation is in itself frequently suppressing to secretory function and an untrained subject cannot be used. The reactions of our subjects may have been conditioned by long laboratory experience so that the very process of intubation restricted the irradiation of impulses, explaining why we failed to demonstrate in any striking way psychic inhibition of acid secretion. Todd and Kuenzel (1929) made fluoroscopic and radiographic studies of the emotional disturbance of gastric patterns and present some evidence in support of this. Fore-knowledge of a contemplated stimulus vitiated the experiment and a psychological stimulus once used could never be employed again.

Cannon (1933) says the changes associated with emotional excitement and vigorous work are similar. Our results suggest that exercise itself is without influence upon the work of the stomach, but that suppression, when it occurs, is due to the irradiation of impulses into the very pathways taken under psychic stress. The mechanism of the emotional inhibition is explained in part by direct action of the sympathetic division of the autonomic nervous system, inhibiting gastro-intestinal muscle tone and the secretion of its glands, and in part by the redistribution of blood which results in splanchnic deprivation and relative anemia of the alimentary tract, thus inhibiting its ability to function (Cannon, 1933). This latter phenomenon is known also to occur in exercise, especially when it is generalized and associated with much sweating. Our findings indicate that when confronted with the necessity of performing strenuous and exhaustive muscular work the beneficent digestive functions are not in abeyance. They carry on in spite of the relative anemia of the organs involved.

It would indeed be disastrous to the efficiency of the gastro-intestinal tract if the vasomotor shifts of exercise were able to inhibit its ability to function under any but the most crucial emergency situations. It requires three or four hours for even a moderate mixed meal to leave the stomach. What goes on in the stomach is of less importance to the organism than the digestive and absorptive functions of the gut. The intestine too must suffer from relative anemia as a result of exercise. During the waking hours the small intestine is probably rarely free of aliment that

must be chemically attacked and then absorbed. Although the stomach is only periodically loaded with foodstuffs, it keeps the intestine much more constantly supplied. To protect the whole alimentary tract from exercise inhibition, muscular work would have to be barred before, after, and between meal times, because when suppression does occur, it outlasts the actual period of effort. It seems to us that the stomach must have what may be called a large margin of safety—the ability to continue working optimally under conditions which seem unfavorable to normal functioning. This thesis should be biologically sound, for processes as fundamental to existence as nutrition cannot be susceptible to too ready change.

CONCLUSIONS

1. The exercise inhibition of the secretion of hydrochloric acid disappears with the repetition of all but extreme grades of work.
2. If the work is so severe that the cardiovascular-respiratory system cannot adjust to it, and it remains always associated with great stress, it continues more or less to inhibit gastric function.
3. The secretory suppression which at first accompanies work under the conditions of our experiments is a part of the total reaction from which a more appropriate response is individuated as the activity becomes automatized.
4. After the response has been conditioned, increasing the severity of the exercise no longer calls forth secretory inhibition.
5. Very severe and exhaustive exercise can therefore be performed without suppressing the acid secreting mechanism of the stomach.

ACKNOWLEDGMENT. Our thanks are due Doctor Tuttle who graciously placed the facilities of the Iowa University Physiology Department at our disposal during the 1934 Mid-West Hockey Tournament which was held in Iowa City. Certain of our observations were made in that laboratory at that time.

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THE EFFECT OF ACUTE ANOXEMIA ON HUNGER, DIGESTIVE CONTRACTIONS AND THE SECRETION OF HYDROCHLORIC ACID IN MAN[1]

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The experiments reported in the preceding paper of this series demonstrated that although at first suppressing, very strenuous exercise may eventually be performed without inhibiting the acid secreting mechanism of the human stomach. Muscular exercise of that severity must be attended by vasomotor adjustments by virtue of which the splanchnic area is subjected to relative anemia. The ability of the acid secreting cells to continue their activity unabated under such seemingly unfavorable circumstances, suggests that the stomach may be an organ relatively insensitive to oxygen want. The work of Van Liere, Crisler and their associates (1930, 1932, 1933, 1934) indicates that such is the case in the dog, for an examination of their published records shows that anoxemia must be exceedingly severe before the digestive movements, hunger contractions or emptying time are significantly affected.

In 1925 Lim, Ivy and McCarthy presented the view that an increase in blood flow will augment the secretion of gastric juice and that all excitatory stimuli act essentially by enhancing the blood flow to the glands. Subsequently Ivy and Farrell (1925) demonstrated that blood supply affects the secretory response of the gastric pouch transplant. In 1927 Lim, Necheles and Ni studied the importance of blood supply to the secretion of the viviperfused stomach and concluded that while increased blood flow may augment secretion already in progress, "gastric secretion occurs within wide limits independently of blood flow." Further, Ivy and Vloedman (1923) found that histamine, which increases the vascularity of the stomach, does not affect its motor conduct, either during periods of hunger or in the interdigestive phase of relative quiescence.

These latter evidences are in agreement with our belief that the splanch-

[1] This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

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nic vaso-constriction of exercise in man has little effect upon gastric function. Since the relative anemia of the visceral area may be assumed to result in a diminution of oxygen supply to the stomach, the problem of gastric function during exercise may be indirectly investigated by studying the behavior of the stomach when the organism as a whole is subjected to oxygen want. The object of this inquiry was therefore to determine the effect of anoxemia upon the acid secreting mechanism of the human stomach and upon its ability to maintain adequate fasting and digestive motility.

METHODS. Anoxic anoxia was produced by low oxygen pressure in the inspired air. The subject rebreathed continuously from a closed circuit in which air of the desired composition was made to circulate, carbon dioxide being absorbed by soda lime. Breathing through the nose was prevented by the application of a nose clip. There were no valves in the rubber mouth-piece which was perforated to admit an air-tight connection for the passage of a Rehfuss tube or balloon. A spirometer included in the circuit was equipped with a marking pen to give a graphic record of respiration. The capacity of the spirometer was 8 liters. Preliminary to use in an experiment, the system was filled with room air, circulated and rebreathed to the desired oxygen tension. The subject was then included in the circuit and a finely adjustable stream of oxygen was steadily admitted to replace that removed to meet the metabolic needs of the tissues. As long as the spirometer level remained constant the income of oxygen balanced outgo. If it seemed desirable to reduce further the oxygen tension during the course of an experiment, the inflow valves were shut and the subject removed oxygen from the mixture then present in the system. The small capacity of the reservoir made it possible to reduce quickly oxygen tension by rebreathing. If the anoxemia became too acute, the subject could be revived instantly by increasing the inflow of oxygen. In 1926 Dreyer had described a method like this in principle.

The secretion of HCl was stimulated by standard test meals of oatmeal gruel or 7 per cent alcohol, or by the hypodermic injection of histamine in a dosage of 0.1 mgm./10 kgm. Observations were commenced 14 to 20 hours after the last meal. The contents of the fasting stomach were aspirated and then the secretory stimulant was administered. Saliva was at no time expectorated because the rebreathing apparatus made this impossible during periods of anoxemia. The presence of the mouth-piece of the rebreather made no appreciable difference in this error after the first few trials. Following the administration of gruel or alcohol, 5 to 10 cc. gastric samples were withdrawn at 15 minute intervals, first mixing the contents by aspiration and re-introduction. After a sample had been withdrawn the tube was cleared by admitting a syringeful of air. When histamine was used as the secretory stimulant the total gastric contents

were aspirated quarter hourly. Free and total acidity were determined by titration with N/10 NaOH using dimethyl-amino-azobenzene and phenolphthalein as indicators. Results are reported in terms of the amount of N/10 NaOH required to neutralize 100 cc. of stomach contents.

Digestive and hunger contractions were studied by recording changes in intragastric pressure, using a small, delicate balloon of condom rubber, a bromoform manometer, and Marey tambour. For the investigation of digestive contractions a meal of 100 cc. of gruel was used. Time was registered synchronously on the kymographs recording intragastric pressure changes and pulmonary ventilation.

After preliminary experimentation during which anoxemia was maintained continuously for as long a time as 109 minutes, it became evident that moderate degrees of oxygen want were without effect upon the phases of gastric function under study. The most intense oxygen want compatible with safety was therefore induced. The duration of the period of anoxemia was limited to one-half hour, and its influence was studied when introduced immediately, 30 and 60 minutes after the administration of gruel, alcohol and histamine. The periods of exposure were never so long when motility was studied because of the shorter and less certain duration of these phases as compared with the acidity cycles. The observations were made on six healthy, young adult women.

The signs of pre-coma anoxic anoxia. Four operators assisted in the conduct of these experiments. The subject sat in a comfortable chair upon an elevated platform, so placed that she might be freely observed in a good light. The director of the experiment sat on a level with the subject and controlled the degree of anoxia by regulation of the oxygen flow. The subject's estimate of her own condition was without value in deciding the limits to which the experiment might be carried. Under acute anoxia critique is conspicuously lacking. The degree of anoxia was judged by close and careful observation of conduct, facial expression, brightness of the eyes, the character of the respiration, color of the skin, mucous membranes and nail beds, and by general clinical signs of distress. Pulse rates were recorded continuously during the anoxemic period. The percentage oxygen in the air circulating in the system was periodically determined.

The subject was maintained on the verge of consciousness. At the detection of unfavorable signs the stream of oxygen was abruptly increased. The subject's condition improved instantly. The promptness of the response to increased oxygen was considered one of the most important indicators of the subject's fitness. She was never held constantly at any given oxygen level. It was adjusted from moment to moment to meet the demands of the organism in toto. The subject was introduced into the circuit when the air contained approximately 10 volumes per cent of oxygen. She was permitted to breathe this until the respirations took on the charac-

ter illustrated in figure 1. The oxygen inflow valves were not until then opened. In a typical half hour experiment the oxygen content oscillated between 7 and 12 per cent depending upon the toleration of the subject. It was enriched above 10 per cent only as necessity demanded and after the revival of the subject was at once carefully reduced again. It was

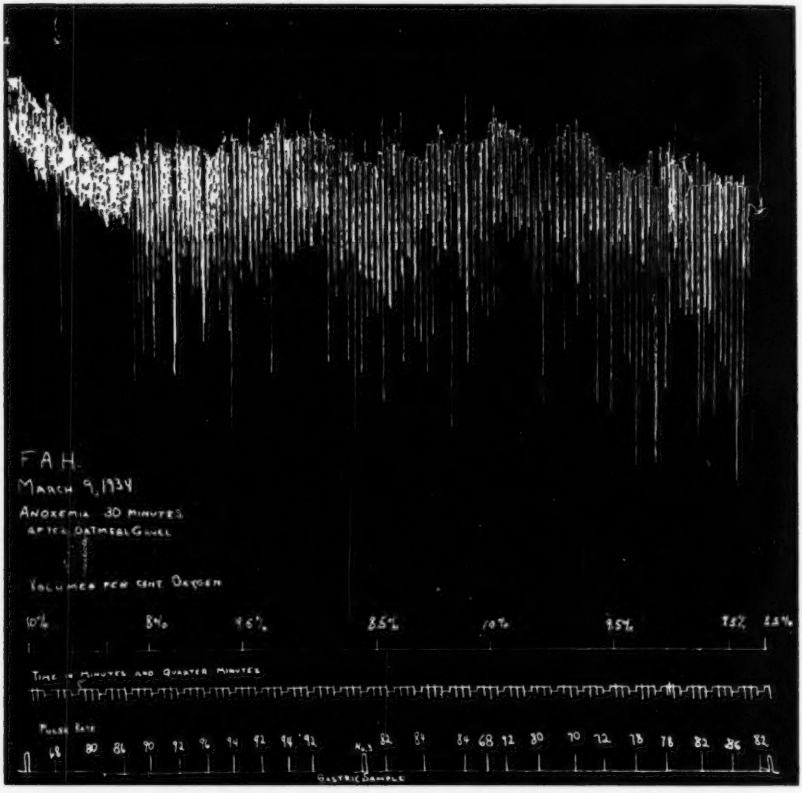

Fig. 1. Kymogram illustrating the respiratory change used as a criterion of pre-coma anoxia.

rarely dropped below 7 per cent and then only momentarily for unconsciousness would have supervened. The character of the breathing, more than any other single factor, was used as a criterion of pre-coma anoxia. The respirations at first increase in depth, less in rate. If the high degree of anoxemia is continued, the respiratory rate soon slows and may be periodic. Breathing is characterized by strikingly deep, gasping inspira-

tions. Expiratory pauses are frequently long, lasting 15 or even 20 seconds. The most profound anoxia in a conscious subject was associated with a respiratory rate of four per minute. Great suppression is illustrated in the kymogram presented. Other constant signs of pre-coma anoxia were uncontrollable restlessness, marked cardiac acceleration and cyanosis.

TABLE 1

The free and total acidity of the gastric juice after the administration of oatmeal gruel, alcohol and histamine to subject F. A. H. under normal conditions and acute pre-coma oxygen want

TIME		OATMEAL GRUEL					ALCOHOL					HISTAMINE							
		Normal		Anoxemia			Normal		Anoxemia			Normal		Anoxemia					
		Mean*	σ	Exp. 9	Exp. 8	Exp. 7	Mean*	σ	Exp. 4	Exp. 3	Exp. 2	Mean*	σ	Exp. 13	Exp. 7	Exp. 12	Exp. 10	Exp. 2	Exp. 11
minutes		*cc. N/10 NaOH to neutralize 100 cc. gastric juice*																	
15	HCl	6	±5	0	9	*0*	27	±9	27	42	*20*	67	±18	76	61	70	73	*50*	*65*
	T. A.	24	±10	18	24	*11*	44	±14	38	50	*29*	85	±18	87	75	81	86	*67*	*77*
30	HCl	30	±18	24	23	*17*	42	±8	40	41	*33*	98	±11	102	100	92	90	*87*	*72*
	T. A.	48	±19	46	38	*27*	62	±10	51	52	*42*	116	±14	111	109	102	100	*99*	*83*
45	HCl	41	±9	23	*30*	28	42	±10	41	*35*	43	97	±10	91	100	*83*	*89*	*93*	94
	T. A.	64	±16	50	*42*	39	64	±10	52	*47*	53	113	±12	100	109	*92*	*99*	*103*	102
60	HCl	44	±5	23	*35*	35	38	±13	32	*55*	38	77	±16	67	*70*	*71*	*88*	*83*	52
	T. A.	63	±10	44	*46*	53	64	±15	45	*65*	47	97	±14	75	*80*	*83*	*98*	*99*	65
75	HCl	43	±10	*30*	32	38	37	±12	*25*	61	37	68	±17	*46*	*71*	49	58	67	50
	T. A.	64	±12	*46*	52	52	63	±14	*36*	72	49	86	±19	*56*	*83*	63	70	95	64
90	HCl	44	±8	*27*	29	37	33	±10	*18*	55	35	64	±19	38	44	32	28	38	50
	T. A.	65	±13	*43*	44	52	59	±14	*33*	68	49	77	±22	50	58	48	33	50	65
105	HCl	43	±13	24	37	34	32	±10	31	57	42								
	T. A.	64	±19	40	49	49	60	±12	45	70	51								
120	HCl	44	±8	30	43	38	34	±8	30	55									
	T. A.	63	±15	45	56	50	59	±6	47	69									

* Arithmetic mean of 8, 21 and 10 experiments respectively.

The acidities of samples obtained during anoxemia appear in italics.

RESULTS. *1. The influence of anoxemia upon the acidity response to gruel, alcohol and histamine.* These observations were made upon three subjects. To establish a base for comparison, 104 experiments were performed under normal conditions. The variability of the healthy stomach in its secretory response to constant stimuli under well controlled con-

ditions is insufficiently appreciated. Forty-seven experiments were performed to establish the limits of variability in the response to the administration of alcohol, 22 to oatmeal gruel, and 35 to histamine. A total of 66 observations was made under oxygen want. In 38 of these the anoxia was of the pre-coma type. The data on subject F. A. H. is presented in table 1. That obtained on S. L. H. and R. E. B. was in all respects similar. To facilitate the interpretation of the findings the variability was calculated in addition to each subject's average acidity response under normal conditions. Thus one may at a glance compare the anoxemic data in table 1 with that obtained at normal oxygen tensions, and weigh them in terms of the standard deviation or spread in the response which occurs even when all controllable factors are rigorously met.

Observations made during the course of anoxemia approximate normal in a way out of keeping with the profound oxygen want induced. Anoxia was never stimulating during the period of administration. It seemed often to be suppressing, but the findings are impossible to evaluate in a quantitative way because of the relative smallness of the change in comparison with the magnitude of uncontrollable variations. The inhibition is never as great as that described in our first observations of the depressing effects of exercise (Hellebrandt and Hoopes, 1934). The ability of the stomach to secrete a juice of good acid content is relatively unimpaired by anoxemia which widely suppresses activity of the cerebral cortex, and which comes close to paralyzing the vital centers.

Graph I, figure 2, presents the secretory data obtained in the course of the experiment illustrated in figure 1. Pre-coma anoxia was continuously maintained for one-half hour with almost negligible effects on gastric acidity. The suppression induced is just below the limits of normal variability. This typefies the usual response. Depressions as pronounced as that in graph II were only rarely met. The secretory response to gruel seemed in general more readily suppressed than that occasioned by either alcohol or histamine. The comparative susceptibility to inhibition when the anoxic intervals were introduced during early, middle or late phases of the secretory response to stimulation cannot be evaluated with confidence because of the low reproducibility of the results. Subject S. L. H. showed a frequent post-anoxemic acidity augmentation as demonstrated in graph III. This was especially apparent when alcohol was used as the secretory stimulant. Graph IV shows the strikingly competent continuance of the secretion of a juice of high acid content during pre-coma anoxia maintained whilst histamine has its most stimulating effect. We noted not infrequently, and especially in subject F. A. H., that anoxia tended to prolong the interval of high acidity which usually ends abruptly 45 minutes after injection when histamine effects are observed at normal oxygen tensions.

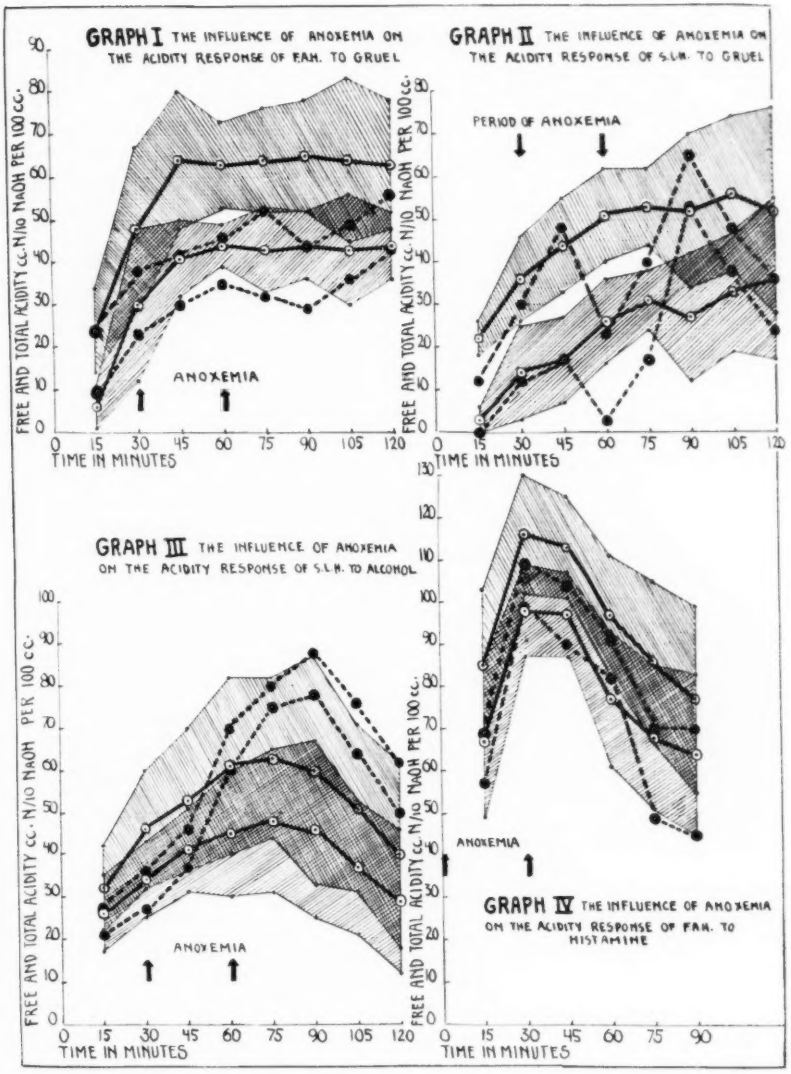
GRAPH I THE INFLUENCE OF ANOXEMIA ON THE ACIDITY RESPONSE OF FAH. TO GRUEL
GRAPH II THE INFLUENCE OF ANOXEMIA ON THE ACIDITY RESPONSE OF S.L.H. TO GRUEL
PERIOD OF ANOXEMIA
ANOXEMIA
GRAPH III THE INFLUENCE OF ANOXEMIA ON THE ACIDITY RESPONSE OF S.L.H. TO ALCOHOL
GRAPH IV THE INFLUENCE OF ANOXEMIA ON THE ACIDITY RESPONSE OF FAH. TO HISTAMINE
FREE AND TOTAL ACIDITY cc. N/10 NaOH PER 100 cc.
TIME IN MINUTES

Fig. 2. Graphs showing the variability of the average acidity response under normal conditions (solid lines) and under the influence of pre-coma anoxia (broken lines). The upper lines in all cases represent total acidity, the lower free HCl. The cross hatched areas map out the standard deviation of the mean free and total acidity respectively.

This may be an expression of the same post-anoxic augmentation evidenced in S. L. H. after alcohol.

2. The influence of anoxemia upon hunger and digestive contractions. Seventy-one experiments were performed, 33 under normal and 38 under anoxemic conditions. Of the latter, 17 were enacted during hunger, 13 during digestion and 8 during the hunger period following a digestive cycle which had itself been subjected to anoxia. There were 3 subjects

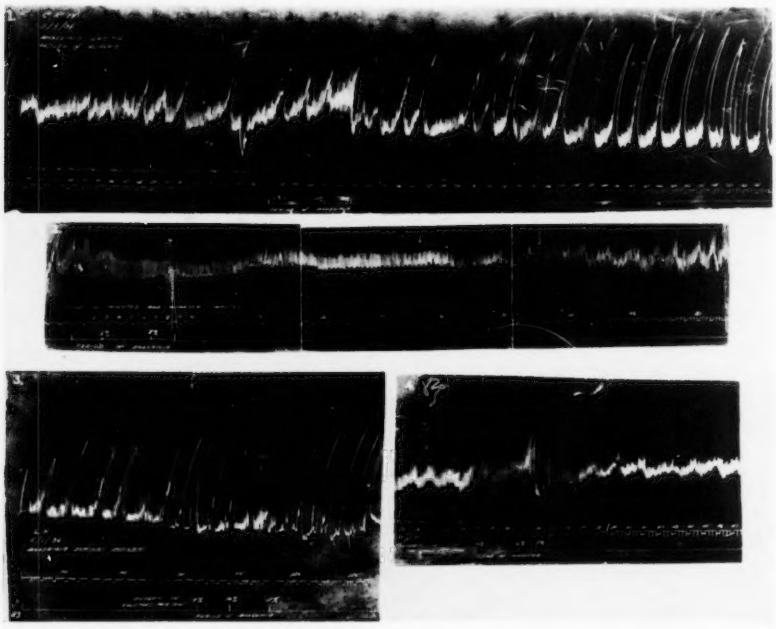

Fig. 3. Kymograms illustrating the effects of pre-coma anoxemia on hunger and digestive contractions.

in addition to those upon whom the secretory studies were made. They contributed 54 of the total observations.

As a whole motility seemed more susceptible to depression than secretion but the degree of change was never marked. We were unable to obtain inhibitions as great as those reproduced in Van Liere's published records on the dog. Examples of the most notable changes induced are presented in figure 3. The effects were for the most part very transitory. Subject C. R. W. was exposed to acute anoxia 9 times. The majority of the observations were made at or below 8 per cent. Because the periods of

exposure were brief, ranging from 5 to 14 minutes and averaging 10, anoxia was pushed to the point of dusky cyanosis. Great air hunger marred the intragastric pressure records. The usual picture was one of gradually developing suppression during anoxia, persisting in augmented form for a few minutes after the return to room air. The average duration of the post-anoxic inhibition was 4.6 minutes in subject C. R. W. She always showed vigorous and prolonged hunger ending in tetanus. As many as 53 hunger contractions of great amplitude occurred during the course of a single hunger period and the longest recorded incomplete tetanus lasted for 13 minutes. When the small meal of gruel was introduced under normal oxygen tension, the tonus rhythm was evident in 20 minutes, well developed in 40, hunger made its appearance in 76 minutes and culminated in tetanus in 120. Anoxemia was introduced three different times during digestion. This insignificantly delayed the reappearance of hunger which occurred at 86, 69 and 107 minutes respectively. Kymogram 1 shows the transitory effect of anoxia imposed during the development of one of these hunger periods following a gruel meal. Hunger had been allowed to develop before the gruel was administered. It terminated in tetanus lasting 8 minutes. The meal was given and in 30 minutes, a sharp, brief exposure to oxygen want. The evanescent effect of this is illustrated in kymogram 4. The tonus rhythm returned and just before these contractions merged into the augmented type characteristic of hunger, the second exposure illustrated in kymogram 1 was made. The disturbance lasted for only a few minutes. Motility of normal amplitude was quickly resumed and typical hunger came on.

J. H. was subjected to anoxia 8 times for intervals ranging between 9 and 14 minutes. The average oxygen percentage was 8. Kymogram 2 shows an unusually prolonged post-anoxemic inhibition, as a result of a 9 minute exposure of a severity which produced a heart rate acceleration from 74 to 102 beats per minute. Motility comparable to that present when anoxia was induced did not recur until after the elapse of 33 minutes. As a result of exposure during which the oxygen fell to 4 per cent and the heart rate jumped to 112, suppression lasted 42 minutes. Evidences of a post-anoxemic augmentation were occasionally seen, although never as clear-cut as those in Van Liere's records. Kymogram 3 illustrates a very moderate augmentation obtained on subject L. H. The anoxemic period was brief but acute for the pulse rate rose from 64 to 116, an increase of 52 beats per minute. The post-anoxemic contractions were of unusual amplitude for the subject concerned.

Discussion. The anoxemia induced in this series of experiments was unquestionably profound. It was of sufficient duration to permit alteration in function if the part concerned is susceptible to oxygen want. We found that cerebral manifestations and cardiac dysfunction occurred be-

fore significant impairment was evident in either secretory or motor behavior of the stomach. This organ is therefore relatively resistant to anoxemia. Though moderately suppressed, it carries on its work in a remarkably competent way under conditions of oxygen want probably rarely exceeded in health or disease. The visceral anoxemia of exercise in which we are chiefly interested, is comparable in duration to that experimentally induced. The findings suggest to us that the paralysis of gastric function which we have observed as an early and transient effect of repeated exercise cannot be due solely to visceral anemia in consequence of the shunting of blood into active muscle and skin regions during the preformance of physical work. The findings are in accord with the hypothesis developed in the preceding paper of this series. When suppression of gastric work does occur, it probably finds its chief cause in direct secretory and motor inhibition by way of the sympathetic division of the autonomic nervous system.

CONCLUSIONS

1. Acute anoxemia of the pre-coma type has relatively little inhibiting effect upon the secretion of hydrochloric acid by the normal human stomach, upon hunger contractions or digestive motility.

2. The exercise suppression of gastric function cannot be explained solely by an anoxemic hypothesis. When it occurs, it is probably due to direct secretory and motor inhibition via the thoracic autonomics.

ACKNOWLEDGMENT. Our sincere thanks are due Dr. W. J. Meek for advice in the development of the method and for encouragment during the conduct of these experiments.

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TONUS RHYTHM IN THE ISOLATED GALL BLADDER AND THE EFFECT OF CERTAIN DRUGS

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Two types of motor activity, which occur in the gall bladders of experimental animals, have been described. One of these is a tonic contraction, which is the result of a sustained muscular activity that produces a marked increase in intravesical pressure, a reduction in size of the vesicle, and the evacuation of its contents. The other is tonus rhythm, which occurs as an alternate contraction and relaxation of the gall bladder with but relatively slight fluctuations in the intravesical pressure.

In our study of the reaction of the gall bladder of sensitized animals to antigen, we observed that tonus rhythm occurred only at certain optimal intravesical pressures. At certain pressures, both the amplitude and frequency of rhythm greatly increased; while at slightly lower or higher pressures, all rhythm was completely abolished. We undertook to study the effect that was produced on tonus rhythm by increasing and reducing the intravesical pressure and to record these changes photographically, with the method hitherto described.

Tonus rhythm of the gall bladder has been studied extensively both in the intact animal and in isolated "in vitro" preparations. Chiray and Pavel, Halpert and Lewis, Ivy and Oldberg, Lieb and McWhorter, Macht, Ravdin and Morrison, Villaret and his associates and Voegtlin and Ivy are but a few of the authors who have studied motor activity in the isolated gall bladder of experimental animals and the manner in which it is affected by the more common drugs. For a complete review of the physiology of the gall bladder the reader is referred to the splendid review by Ivy. We have also studied the effect of certain of the more common drugs on the amplitude and frequency of rhythm in the isolated gall bladder of guinea pigs and dogs. Although these pharmocologic effects are generally known, there are contradictory statements in the literature, and methods of recording and reproducing these effects have not always been satisfactory.

METHODS. Adult guinea pigs and a few dogs were used. The animals were killed by infiltrating the groin with novocain and then severing the femoral artery. The gall bladder and biliary tract were rapidly removed, placed in Tyrode's solution, and a small glass cannula was tied into the

cystic duct. All bile and adjacent hepatic tissue were removed from the preparations, because we have found that they interfere with muscular activity of the vesicle. The gall bladder was immersed in a bath of the Tyrode's physiologic solution (50 cc. for the gall bladders of guinea pigs and 125 cc. for gall bladders of dogs) which was kept at a constant temperature of 38.5°C. Oxygen was supplied freely.

The inlying cannula was connected by means of a rubber tubing with a T-tube, one arm of which was joined to a graduated buret, while the other led to the water manometer, which was arranged to record changes in pressure photographically. We used a manometer of small bore, of the sort ordinarily employed for determining the pressure of the spinal fluid. One centimeter of the manometer contained but 0.2 cc. of fluid, so that slight variations in pressure without significant changes in the volume of the gall bladder were recorded.

When testing for the effect of a drug on tonus rhythm, we withdrew 1 cc. of fluid from the bath, added the drug to this fluid, and then returned it to the bath. In this way, we derived a more uniform dilution of the drug, and the concentration which is indicated is always the concentration in the volume of the bath solution. Every precaution was taken to avoid any accessory influence upon the viscus.

In some experiments a strip of duodenum was suspended in the bath, together with the gall bladder, so that the reactions of duodenum and viscus were recorded simultaneously. A lever, which recorded elapsed time, was fixed in front of the manometer so that its movements were likewise recorded on the revolving sensitized paper.

EXPERIMENTAL OBSERVATIONS. *A. Intravesical pressure and tonus rhythm.* The accompanying illustration (fig. 1) is a photograph of a record of a rhythmically contracting isolated gall bladder of a guinea pig. By means of the buret, the gall bladder had been slowly filled until rhythmic contraction of the viscus began. Rhythm ordinarily began when the intravesical pressure was 25 or 30 mm. of water, although occasionally a gall bladder was encountered which contracted rhythmically when the intravesical pressure was 20 mm., while some did not demonstrate rhythm when this pressure was less than 50 mm. of water. The frequency of rhythm varied in the normal gall bladder from one and a half to two contractions per minute, and the amplitude of rhythm ranged from 30 to 40 mm. Very often we encountered in these gall bladders a secondary rhythm, which was superimposed upon the first, usually at the height or peak of the contraction wave (figs. 1, 10 and 11).

Since tonus rhythm appeared to be dependent on optimal intravesical pressures, we studied the onset of rhythm in relation to a gradually increasing intravesical pressure. We added from the buret to the manometric system, including the isolated empty vesicle at 0 pressure, 0.2 cc.

of fluid every five minutes, and recorded the changes in pressure, which were indicated on the manometer after each addition of fluid. In this manner, we recorded not only the pressure in the vesicle but also the pres-

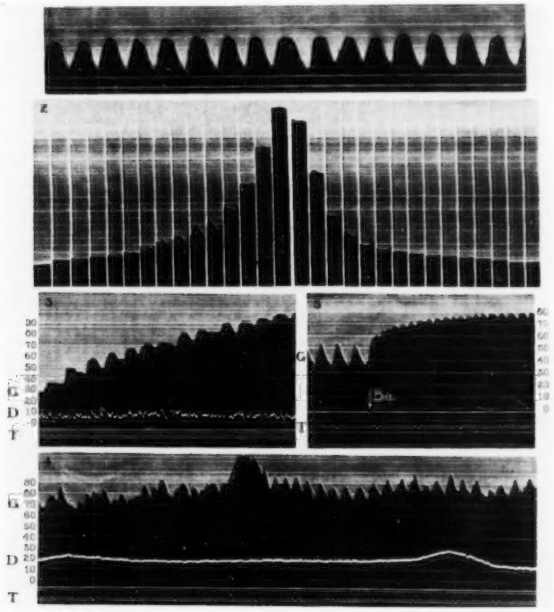


Fig. 1. Record of rhythmically contracting, isolated gall bladder of guinea pig.

Fig. 2. Record of intravesical pressure. The vertical white lines represent five minute intervals between successive additions, to the content of the gall bladder, of 0.2 cc. of fluid. When a pressure of 230 mm. of water had been reached, 0.2 cc. of fluid was withdrawn every five minutes. The intervals between horizontal lines each represent a pressure of 10 mm. of water.

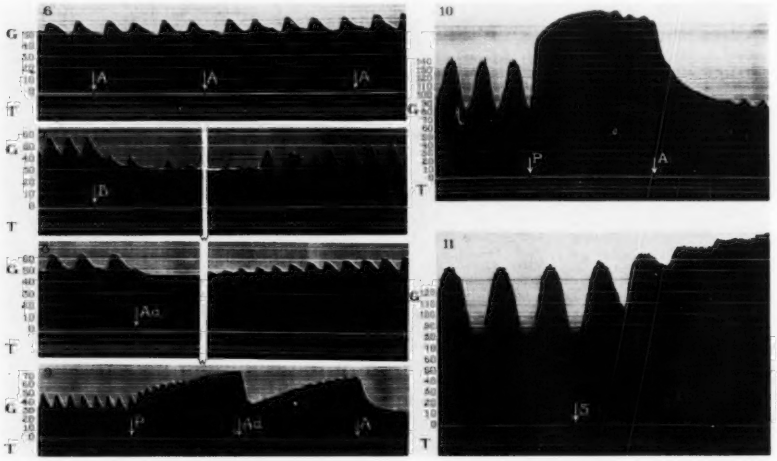
Fig. 3. Spontaneous tonic contraction of gall bladder, of long duration. *G* represents gall bladder; *D*, strip of duodenum and *T*, time marker (interval between broad marks, 1 minute).

Fig. 4. Spontaneous tonic contraction of gall bladder, of short duration; at its height, the complex contraction reaches a pressure of 118 mm. of water. The initials have the same significances as in figure 3.

Fig. 5. The effect, on rhythmic contraction of the gall bladder, of adding barium chloride, 1 to 125,000, to the bath in which the viscus was suspended. *Ba* represents the point in the experiment when the chemical was added. *G* and *T* have the same significance as before.

sure at which tonus rhythm began and when all rhythmic activity ceased. After filling the vesicle to overdistention, a comparable amount of fluid was withdrawn every five minutes and another forty-five second exposure

was made of the manometric fluid level. Fluid was thus withdrawn until the pressure recorded was again zero. Consequently, at the end of the experiment a complete record was available of gall-bladder activity during both the increase and decrease of intravesical pressure (fig. 2). This experiment was repeated a number of times with the same gall bladder and with gall bladders of several guinea pigs, and the results were essentially constant. Under the conditions of our experiment, tonus rhythm began at pressures hovering around 20 to 25 mm. It reached a maximal ampli-



Figs. 6 to 11 inclusive. The effects on rhythmic contraction of the gall bladder, of adding various substances to the bath in which the viscus was suspended. The gall bladders represented in figures 6 to 9 inclusive were from guinea pigs; those represented in figures 10 and 11 were from dogs. In each figure *G* represents gall bladder and *T* time, as before. *W* represents the word "washed", and means that the fluid of the bath was changed twice. The other initials and syllables represent points in the experiments when the various substances were added: *A*, atropine, 1 to 500,000; *B*, autogenous bile, 1 to 750; *Ad*, adrenalin, 1 to 500,000; *P*, pilocarpine, 1 to 500,000; *S*, strophanthin, 1 to 400,000.

tude at pressures from 50 to 70 mm., and usually disappeared or was at a minimum at pressures above 100 mm.

Tonus rhythm, we believe, is more dependent on intravesical pressure than it is on volume, for gall bladders vary in their pressure response to given amounts of fluid. The addition of given amounts of fluid from the buret produced varying amounts of pressure in different gall bladders, but rhythm, however, did not occur until certain pressures were attained. Pressure is dependent on muscle tonus, or on the resistance the vesicle offers to distention. Since muscle tonus in the gall bladder varied in

different animals, and even in the same gall bladder at different times, the resistance to a known volume of fluid likewise varied. Rhythmic activity was remarkably constant, however, when the intravesical pressure ranged between certain limits.

Tonus rhythm occurred as a contraction and relaxation over and above the resistance offered by the vesicle to distention. Ivy and Oldberg observed that rhythmic contractions, which amounted to as much as 2.5 cm. of bile, were superimposed on the tonic contraction. Whenever we added small amounts of fluid to the manometric system from the buret, the gall bladder offered a certain resistance, but when rhythm occurred it appeared over and above the tonus level. When rhythm came to rest, it ceased at those pressures at which it began rather than at the height of its amplitude.

Both the frequency and amplitude of gall-bladder rhythm are dependent on the pressure within the vesicle. When the intravesical pressure was increased, the amplitude of rhythm was invariably decreased, although the frequency of the rhythmic contractions increased. From a frequency of one rhythmic contraction per minute at a pressure of 30 mm., we often encountered ten contractions and relaxations per minute at a pressure of 100 mm. The amplitude of these waves, however, was always appreciably less.

Occasionally, a spontaneous tonic contraction of the gall bladder occurred without any apparent cause and continued for some time without appreciable effect on tonus rhythm (fig. 3). In the case illustrated, nothing had been added to the bath, there was uninterrupted activity of the duodenal strip, and yet the gall-bladder pressure rose from 30 to 115 mm. of water, with but slight change in either the frequency or amplitude of the rhythm. On the other hand, spontaneous tonic contractions may be of short duration (fig. 4) and may completely abolish all rhythmic activity.

B. The effect of certain drugs on tonus rhythm. Although many studies have been made upon the reaction of the musculature of the gall bladder to drugs, we have employed the photographic method to record the effects produced within the viscus.

Histamine had a most potent effect on the gall bladder of the dog and guinea pig. In minute quantities, it produced an almost instantaneous tonic contraction and completely abolished rhythm. In dilute concentration, the intravesical pressure rose from 40 mm. to 200 mm. of water. When the bath to which the histamine was added was changed, and fresh Tyrode's physiologic solution was added, the tonic contraction ceased, relaxation ensued, and tonus rhythm again occurred at the usual pressure level. Following a tonic contraction which was induced by histamine, there invariably was an improved amplitude and frequency of rhythm after changing the fluid in the bath. Ivy and Oldberg observed that a small dose of "cholecystokinin" would increase the amplitude of rhythmic contractions.

Atropine, 1 to 500,000 in our hands, was without effect on either the muscle tonus or the activity of a rhythmically contracting gall bladder (fig. 6), although by some observers (5, 6, 7, 8) it is said to cause relaxation of the gall bladder of the cat, dog and monkey. However, when a marked tonic contraction had been induced, the addition of atropine caused a prompt relaxation (figs. 9 and 10).

Conflicting reports of the effect of adrenalin on the isolated or intact gall bladder have been made. Voegtlin and Ivy could not demonstrate any constant effect with the intravenous administration of enormous doses. When we added adrenalin in a concentration of 1 to 500,000 to the solution in the bath, there was a decline of but 5 mm. in the intravesical pressure, although rhythm was completely abolished (fig. 8). Normal muscle tone and gall-bladder rhythm were restored after the preparation was washed and the fluid for the bath was added. During tonic contraction induced by pilocarpine, adrenalin induced a prompt but temporary relaxation of the gall bladder (fig. 9) of the guinea pig, but did not have any effect on the contracting isolated gall bladder of the dog.

Pilocarpine invariably caused a marked contraction of the isolated contracting gall bladder of either the dog or the guinea pig. In a concentration of 1 to 500,000, there was a gradual increase in tonic contraction and a decrease in the amplitude of tonus rhythm (fig. 9). In the dog (fig. 10) there was an increase of 110 mm. in the intravesical pressure, and a complete cessation of all rhythmic activity.

Barium chloride in a concentration of 1 to 125,000 induced an immediate and sustained increase in tonic contraction of the gall bladder, a decrease in the amplitude of rhythm of the contraction, but an increase in its frequency (fig. 5).

Autogenous bile (5) induced a marked effect on muscle tonus and rhythmic activity of the gall bladder. When added to the bath, in a concentration of 1 to 750, there was an immediate decrease of 10 mm. in intravesical pressure, an interruption and a final cessation of all rhythmic activity (fig. 7). Ivy and Oldberg observed no effect on the gall bladder when dilute bile or 1 per cent solutions of bile salts were injected intravenously.

Amorphous strophanthin, in a concentration of 1 to 400,000, had a marked effect on tonus rhythm of the isolated gall bladder of the dog. Intravesical pressure increased approximately 100 mm. of water and all rhythm was essentially abolished (fig. 11).

SUMMARY

Tonus rhythm has been studied in the isolated gall bladder of the dog and the guinea pig. Records of this rhythm have been made photographically on sensitized bromide paper. The isolated gall bladder of the dog,

when suspended in warm Tyrode's physiologic solution, demonstrated rhythmic activity with an amplitude of 60 mm., which ranged from 80 mm. to 140 mm. of water. The isolated gall bladder of the guinea pig demonstrated rhythmic activity with amplitudes ranging from 20 to 40 mm. of water, at pressures which varied from 20 to 50 mm. of water in different animals.

Tonus rhythm is definitely related to intravesical pressure. When fluid was added slowly to the empty gall bladder of the guinea pig, which was suspended in a physiologic solution, tonus rhythm began when the gall bladder pressure was 20 to 25 mm. of water. Rhythm ceased or was at a minimum at pressures of more than 100 mm. of water. Tonus rhythm occurred as a contraction and relaxation over and above the resistance offered by the vesicle to distention.

Histamine produced an almost instantaneous contraction of the gall bladder and completely abolished rhythm. Intravesical pressures rose from 40 mm. to 200 mm. of water. Atropine did not affect either tonus rhythm or the tonic contraction of a rhythmically contracting gall bladder. In the presence of marked tonic contraction, however, atropine induced a prompt relaxation of the gall bladder. Adrenalin, in a concentration of 1 to 500,000, induced a slight decrease in tonic contraction and a cessation of rhythmic activity. Adrenalin induced a prompt but temporary relaxation of the gall bladder during a tonic contraction caused by pilocarpine. Pilocarpine always caused a marked contraction of the isolated gall bladder of either the dog or guinea pig. Barium chloride induced an immediate and sustained increase in the tonic contraction of the gall bladder. Autogenous bile induced a relaxation of the rhythmically active gall bladder with cessation of rhythm. Strophanthin increased tonic concentration and abolished rhythmic activity.

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NEUROGENOUS ACTIVATION OF CILIATED EPITHELIUM

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The major conclusion that nerves modify the rate of ciliary movement in the palate of the frog, first advanced by McDonald, Leisure and Lenneman (1), has received confirmation by Pohle (2), Seo (3) and Lucas (4), but factual and experimentally derived data reported by each of these later workers has brought into question the validity of some procedures and the conclusions resulting therefrom upon which the original hypothesis was founded. The twofold purpose of the present study has been to seek with the aid of methods more recently developed for study of ciliary activity, support for the major conclusion already advanced and to identify the nerve components initiating the ciliary response.

McDonald, Leisure and Lenneman consider that the cilia of the frog's palate are continuously active, that electrical stimulation of the sympathetic trunk and certain autonomimetic drugs induce acceleration while stimulation of the brain and the action of certain other drugs slow the rate of movement. Their base line of normal activity determined by the rate of passage of particles across the surface was called into question when the reflection method was developed (5), which method eliminates the use of particles or fluids upon the ciliated surface and which demonstrates that such fluids or particles are, in themselves, stimulating agents and finally that in the frog's palate the unstimulated cilia are completely quiescent.

METHODS. The different phases of the investigation involved the following procedures:

Stimulation of the sympathetic trunk. An exsanguinated unpithed frog fastened to a movable platform, with the jaw retracted, was opened to expose the thoracic sympathetic trunk. Stimulating electrodes on the sympathetic trunk at the level of 1st and 3rd or 4th spinal nerves carried single induction shocks. The intensity of the shock was changed by varying a resistance in the primary circuit.

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[2] The preparation of the manuscript was completed in the Department of Zoology, State University of Iowa.

Transection of cranial nerves. The 5th, 7th, 9th and 10th cranial nerves and the cranial sympathetic trunk were severed intracranially as shown in figure 1, after removal of the skull plate. Swabs made on toothpicks aided in the operation and a Graefe's cystotome served as a suitable neurotome. The operations were performed only on one side and the opposite side as a control indicated any extensive injury. Operations on 17 out of 25 frogs were successful. Stimulation of the tongue and the resulting response by the palatal cilia was used to determine the effect of nerve ablation upon the reflex pathway.

Nerve-cilia preparations. The method first devised by Seo (3) was modified to the extent illustrated in figure 2. The tibial nerve of the leg freed of tissue to the level of the foot and the palatine nerve was laid across the stimulating electrodes. The electrode adjacent to the responding tissue was grounded to prevent possible spread of current. A Harvard inductorium whose primary was connected with an interruptor set to give approximately three stimuli per second and with a variable resistance of 1 to 999 ohms produced stimuli of threshold intensities. The amount of resistance served as a measure of stimulating current and threshold values were compared by the values obtained. The threshold responses for the nerve-muscle and nerve-cilia preparations were accepted as the first muscular twitch in the toe of the foot and activation of the palate cilia.

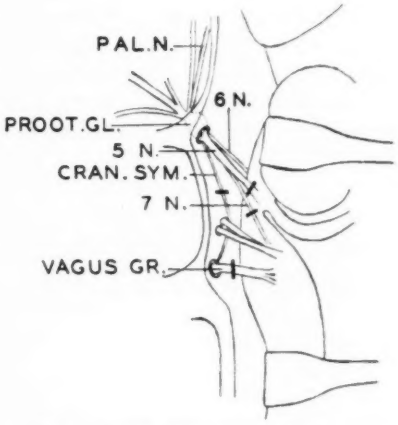

Fig. 1. Cranial and sympathetic nerves. Arrangement of 5th to 10th cranial nerves and cranial sympathetic trunk within the cranial cavity of the common green frog. The 5th, 6th, 7th and sympathetic enter the proötic ganglion from which arises the palatine nerve. The solid lines across the nerves indicate the regions at which they were transected.

Through the kindness of Prof. George H. Bishop it was determined on the cathode ray oscillograph that the first twitch of the toe or foot muscles corresponds to the threshold of the A potential wave.

Comparison of nerve-fiber size distribution. The palatine and tibial nerves of the bullfrog were prepared for osmification of the myelin by first perfusing with a solution of 0.4 per cent NaCl and 0.5 per cent $MgSO_4$; the resulting edema separates the nerve fibers and this followed by perfusion with $HgCl_2$ saturated in the above salt solution, enhances fixation of the

fibers in their normal form. The nerves were dissected out, immersed in 2 per cent osmic acid for from 24 to 36 hours and sectioned following double imbedding according to Peterfi. The fiber diameters were measured according to the method of O'Leary, Heinbecker and Bishop (6) and plotted in the manner described by them and by Douglass, Davenport, Heinbecker and Bishop (7).

OBSERVATIONS AND CONCLUSIONS. *Stimulation of the sympathetic trunk.* McDonald, Leisure and Lenneman (1) found that electrical stimulation of the sympathetic trunk accelerated the cilia of the palate and concluded that activator fibers have a pathway through this system.

Repetition of this experiment failed to give these results after a significant possible source of error was taken into account, namely, that the retraction of the jaw brings the afferent nerves of the tongue and adjacent tissues in proximity to the electrodes stimulating the sympathetic trunk.

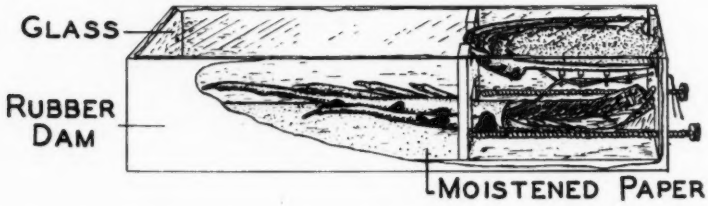

Fig. 2. A sketch of the moist chamber used to obtain values of threshold stimuli to the tibial and palatine nerves. The electrode to the left is grounded, the remaining two carried the electrical stimulus. Wooden pegs were used to fasten the tissues in place.

When such occurs the strength of stimulus adequate to activate the cilia of the palate is about the same as when the palatine nerve is stimulated. The explanation for this result is evident from the work of Seo (3) who demonstrated that a closely integrated reflex exists between points of stimulation on the tongue and correspondingly located points on the palate. Possible error from this source was eliminated by elevating the jaw. This changed the previous apparent threshold value in that the relative current strength required to activate the cilia increased about 10 to 15 times and was about 150 to 225 times greater than that required to produce skeletal muscle contractions by stimulation of the tibial nerve. Moreover, when the shock is made sufficient to activate the cilia, the electrodes and sympathetic nerve being free from adjacent tissue, contractions are induced in the surrounding thoracic muscles, indicating that the spread of current has been apparently responsible for the response and not activation through the sympathetic nerves.

Transection of cranial and sympathetic nerves. The probable absence of accelerator fibers in the sympathetic was subjected to further experimentation utilizing the tongue-cilia reflex described by Seo (3). Two pathways for the reflex are conceivable; one, through the afferent fibers from the tongue to the brain and from there to a sympathetic trunk by way of a spinal nerve, thence to the proötic ganglion and finally along the palatine nerve; two, from the afferent fibers of the brain upward to one of the cranial nerves leading to the proötic ganglion and to the palatine nerve. Seo, unaware of McDonald, Leisure and Lenneman's work, suggested the latter pathway but his experiments in which the nerves were cut outside the cranial cavity did not preclude the first possibility.

The results presented in table 1 based on transections of nerves shown in figure 1, seem to justify the interpretation that no reflex occurs when the 9th nerve is cut because the afferent pathway from the tongue is severed, that the cranial sympathetic trunk is not involved in the reflex pathway

TABLE 1

Effect upon the tongue-palatal cilia reflex of cranial and sympathetic nerve transection

NUMBER OF FROGS	NERVES TRANSECTED	CILIARY RESPONSE ON THE PALATE AFTER STIMULATION OF THE TONGUE
1	9th and 10th cranial	−
1	5th, 7th and cranial sympathetic trunk	−
7	cranial sympathetic trunk	+
1	5th and 7th cranial	−
5	5th cranial	+
2	7th cranial	−

and that the motor afferent neurons to the palatine nerve have their cells of origin in the brain stem and pass peripherally along the seventh cranial nerve.

Cessation of ciliary activity. The reflection method, eliminating the use of stimulating particles or fluids, reveals that the cilia are normally inactive but this does not preclude the possibility that this state is brought about by nerve action. The following facts, however, would indicate that nerves play no part in stopping the cilia but rather that the cessation of movement is an inherent property of these ciliated cells: 1. After a local or a reflex stimulus has passed, the return to the quiescent stage, *in situ*, is gradual, covering a period of several seconds to a minute or more and some cilia come to rest long before others. This is in contrast to the activation response which is known to be initiated by nerves, in that all the cilia, practically simultaneously, within a fraction of a second, attain their maximum velocity. 2. When the palate mucous membrane is removed

and spread, epithelial surface uppermost without a cover glass or the addition of Ringer's solution, the cilia behave in exactly the same way as they do in the intact animal as far as *local* reactions are concerned, that is, they are at rest until stimulated and return to rest some time after the stimulating agent has been removed. The response is, therefore, independent of the central nervous system.

TABLE 2

Ratio of threshold stimuli for nerve-muscle and nerve-cilia preparations

TIBIAL NERVE-FOOT MUSCLE PREPARATION		PALATINE NERVE-CILIA PREPARATION		RATIO OF THRESHOLD VALUES
Number of determinations for each nerve	Average resistance in primary circuit in ohms	Number of determinations for each nerve	Average resistance in primary circuit in ohms	
		Green frog*		
2	420	1	31	1 to 13.5
2	447	1	40	1 to 11.2
8	460	8	24	1 to 19.3
13	484	10	26	1 to 18.6
9†	768	10	74	1 to 10.4
5†	600	3	43	1 to 14.0
Average				1 to 14.6
		Bullfrog‡		
3	317	2	19	1 to 16.7
3	287	2	17	1 to 16.8
2	253	4	19	1 to 13.1
4	332	7	29	1 to 11.4
5	365	11	40	1 to 9.1
1	253	1	14	1 to 18.1
10	224	9	18	1 to 12.4
13	697	9	39	1 to 17.9
6	333	4	20	1 to 16.6
4	402	5	18	1 to 22.3
Average				1 to 15.4

* Threshold values obtained separately for palatine and tibial nerves.

† Data for the right and left nerves of the same frog.

‡ Threshold values obtained with both nerves across the electrodes.

Allocation of cilio-accelerator fibers to the autonomic system. Evidence was sought to determine whether the accelerator fibers belong to the somatic motor or autonomic systems by comparing the strength of threshold stimuli applied to the palatine and tibial nerves which would, respectively, elicit ciliary movement and twitching of foot muscles. The moist chamber employed is illustrated in figure 2 and the method already described.

The tibial and palatine nerves were stimuated separately in the first six experiments carried out on the common green frog. The threshold values and their ratio to each other are given in table 2. Possible error due to differences in size of the two nerves is somewhat counter-balanced by the necessary inclusion of more connective tissue with the palatine nerve which tends to make the tissue resistance equal to that of the tibial nerve. Later improvements in procedure involved the use of the bullfrog with its longer palatine nerve and both tibial and palatine nerves were on the electrodes while the value for each was being determined.

In the green frog strength of stimulus to the palatine nerve necessary to activate the cilia averages 14.6 times greater than is required to produce

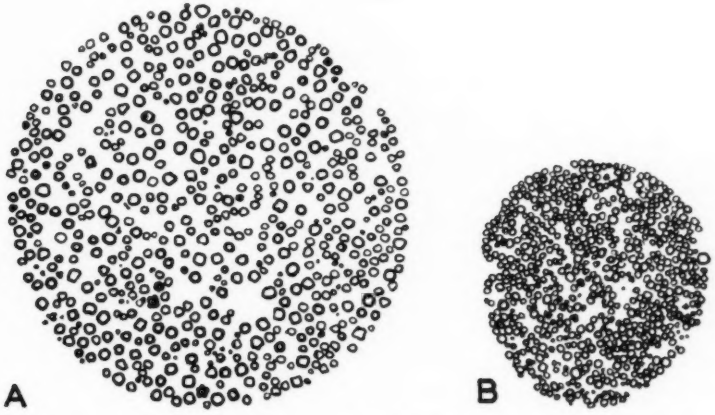

Fig. 3. Magnification of each nerve, 136×.[3] A. Cross section of tibial nerve of bullfrog; 732 myelinated fibers. B. Cross section of palatine nerve of same frog; 663 myelinated fibers.

slight contractions of the muscles of the foot when the tibial is stimulated. The variation in ratio is from 1–10.4 to 1–19.3. The average ratio for threshold stimuli producing these two responses in the bullfrog is 1–15.4 with variations from 1–9.1 to 1–22.3.

It is on the basis of recent publications by Bishop, Heinbecker and O'Leary (8), and after a reinvestigation of certain relative threshold values given by Heinbecker (9) had been made, that the cilio-accelerator fibers were assigned to the autonomic system. Heinbecker reports for frogs, that to the A potential wave, the B_2 has a threshold about 26 times greater.

[3] The edematous and enlarged condition of the tibial nerve in comparison with the palatine nerve is due to the method of preparation and in this case, the latter did not receive as much of the perfused salt solution.

Bishop redetermined the ratio of A and B_2 thresholds in the bullfrog sciatic nerve, in view of the fact that the value of such a ratio depends on the duration of the stimulus, and this in turn depends on the size of the nerve trunk and distance between electrodes, as a part of the resistance of the circuit. Using condenser charges as stimuli, a 0.05 mf. condenser gave a ratio of A to B_2 thresholds in the tibial nerve of the bullfrog in one experiment of 13, in a second of 15, and a 0.02 mf. condenser a ratio of 1 to 20, and with smaller condensers the B_2 wave was not stimulated without unreasonably

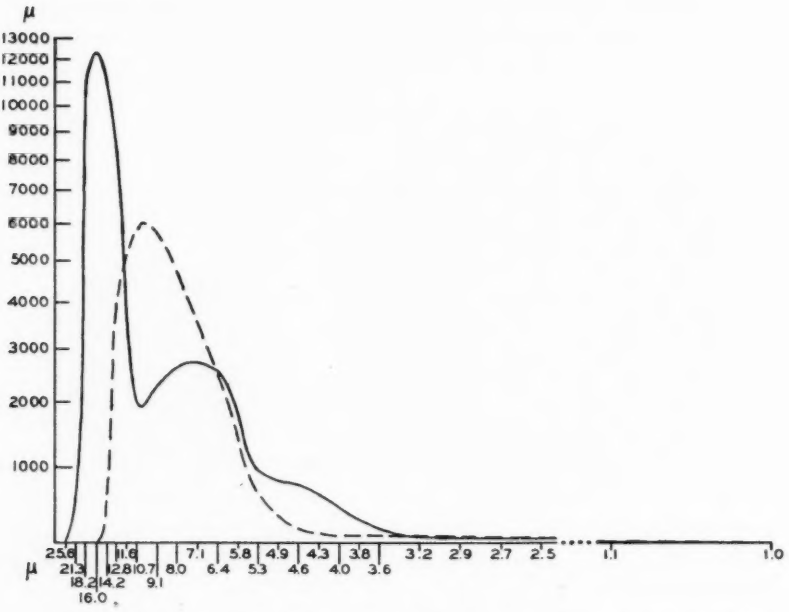

Fig. 4. The distribution of nerve fiber area plotted against a base line of fiber diameter. Tibial nerve is represented by the solid line; the palatine nerve by a broken line.

high voltages. In preparations of the palatine nerve usually employed, where the electrodes were necessarily close together and the resistance correspondingly low, the induction shock must have been prolonged as compared to the usual conditions of sciatic stimulation, which would decrease this ratio. Bishop also determined that the first muscular twitch on the tibial nerve is identical with the threshold for the A potential, making valid a comparison with their results based on oscillographic records.

The threshold ratio of 1:15 for functional responses, falls in the range of 1:13 to 20 obtained for the A:B_2 nerve potentials and on this evidence it is suggested that the cilio-activator fibers belong in the B_2 potential group and on the basis of studies by Bishop, Heinbecker and O'Leary (8), that they belong to the autonomic system rather than to the somatic motor system.

This conclusion, in conjunction with the ones already given would place these fibers in the bulbo-autonomic system having their cells of origin in the brain and coursing through the seventh cranial nerve.

Size distribution of palatine nerve fibers. The palatine nerve is particularly favorable for testing conclusions of Bishop, Heinbecker and O'Leary that the function potential group and fiber size may be correlated. The palate lacks skeletal muscle and presumably contains only sensory fibers and those belonging to the autonomic system innervating ciliated and goblet epithelial cells and blood vessels. The diameters of myelinated fibers in the tibial and palatine nerves illustrated in figure 3 were measured and their areas plotted according to fiber size (fig. 4). The tibial nerve with its somatic motor and sensory components shows a curve with peaks at 16 mu and 7 mu, both of which according to Douglass, Davenport, Heinbecker and Bishop (7) fall within the range of A and B_1 potentials. The sensitive palatal mucosa with its various sensory endings is innervated by fibers falling within a single curve, with a peak at 10.5 mu. That sensory fibers should correspond to a large extent with the second wave of the tibial nerve fibers corroborates the conclusion of Bishop, Heinbecker and O'Leary that many somatic sensory fibers are located in the B_1 or in the last part of the A wave.

The B_2 potential wave for mammalian nerves is correlated with fiber size of 1 to 3.3 mu. Only 10 fibers in the tibial nerve and 30 in the palatine nerve of the bullfrog measure less than 3.6 mu in diameter. In view of the fact that the A:B_2 threshold ratio is less in the frog than in mammals it seems probable that the diameter of fibers belonging to this potential group are larger than in mammals and as represented in figure 4, may include more than the flattened tail for the curve for the two nerves.

The neuro-ciliary relationships which have been discussed are limited to the green frog, Rana pipiens and to the bullfrog, R. catesbiana. An attempt to extend these observations to turtles and ducks has shown that in the trachea of these animals nerves exert no regulatory influence over ciliary action (Lucas and Douglas, 10).

I wish to thank Prof. George H. Bishop for many helpful suggestions during the progress of these experiments and for his criticism of the manuscript.

SUMMARY

1. Fibers in the palatine nerve of the green frog and the bullfrog are responsible only for activation of ciliary movement and not for its inhibition. The cessation seems to be an autonomous inherent property of these cells.

2. The sympathetic nerve trunk does not carry any of the accelerating fibers to the ciliated epithelium of the palate.

3. The accelerating fibers have their cells of origin in the brain and most of the fibers pass out the 7th cranial nerve.

4. Since the stimulation threshold for the cilio-accelerator fibers corresponds to the threshold for the B_2 potential group of the tibial nerve it is concluded on the basis of work by Bishop, Heinbecker and O'Leary that the cilio-activator fibers belong to the autonomic system and in view of other facts already mentioned that they belong to the bulbo-autonomic or parasympathetic portion of that system.

5. Confirmation is given that the sensory fibers are distributed largely in the B_1 potential group.

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THE INFLUENCE OF PITUITARY GROWTH HORMONE ON THE PHOSPHATASE ACTIVITY OF BONE AND KIDNEY[1]

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Since the pituitary growth hormone and the enzyme phosphatase are thought to be closely associated with bone growth, the question arose as to what influence, if any, daily injection of the hormone would have on the phosphatase activity of the bones.

Phosphatase is known to occur much more abundantly in growing bone than in adult bone. On the other hand, kidney phosphatase is said to increase with age within certain limits. The primary question concerned in this study was whether the renewed bone growth of "weight-plateaued" rats, resulting from the administration of pituitary growth hormone, is accompanied by an increase in the phosphatase activity of the bones. The fact that the epiphyses of the rat do not close with the cessation of growth makes this animal peculiarly well suited for this type of work. Symmetrical skeletal growth, instead of acromegaly, results from the administration of pituitary growth hormone. Presumably the changes in any long bone may be taken as representative of those in the other long bones of the body.

Sixteen adult female rats were used. Before beginning the injections the animals were weighed at regular intervals to insure their being "weight-plateaued". They were then divided into pairs of about equal weight and each pair placed in a separate cage. Of each pair one animal received the growth hormone and the other was used as a control. The experimental animals were injected twice daily for 15 to 32 days with 0.5 cc. of a preparation of anterior pituitary growth hormone. After beginning the injections, all the animals were weighed daily for the first 10 days and three times a week thereafter.

Two animals were sacrificed after 15 days, ten after 26 days, and four after 32 days. The animals were killed with chloroform, the kidneys

[1] The authors wish to acknowledge their indebtedness to E. R. Squibb and Sons for supplying the hormone preparation.

[2] Fellow in Medicine of the National Research Council.

immediately removed, weighed, extracted with water by the method of one of the authors (1), and phosphatase determinations made according to the method of Jenner and Kay (2). The left femur of each animal was removed intact, thoroughly cleaned, and weighed in a weighing bottle on an

TABLE 1

The effect of repeated subcutaneous injection of pituitary growth hormone on the body, femur and kidney weights, and on the phosphatase activity of the femurs and kidneys

ANIMAL NO.	NO. DAYS INJECTED	WEIGHT AT BEGINNING OF INJECTION	WEIGHT AT SACRIFICE	GAIN OR LOSS IN WEIGHT	WEIGHT OF LEFT FEMUR	PHOSPHATASE ACTIVITY OF FEMUR PER 25 CC. OF EXTRACT (EQUIV. TO 1 GM. OF TISSUE)*	TOTAL PHOSPHATASE ACTIVITY OF ENTIRE WATER EXTRACT OF FEMUR	WEIGHT OF COMBINED KIDNEYS	PHOSPHATASE ACTIVITY OF KIDNEYS PER 25 CC. OF EXTRACT (EQUIV. TO 1 GM. OF TISSUE)*	TOTAL PHOSPHATASE ACTIVITY OF ENTIRE WATER EXTRACT OF KIDNEYS	REMARKS
		grams	*grams*	*grams*	*grams*			*grams*			
1	Control	272	275	+3	0.719	7.9	5.68	1.957	29	57	
2	26	270	317	+47	0.782	6.9	5.42	2.061	25	51	
3	Control	262	256	−6	0.743	6.3	4.68	2.010	37	74	
4	26	272	331	+59	0.760	5.9	4.48	2.394	32	77	
5	Control	266	282	+12							Tumors†
6	26	257	324	+67	0.743	6.1	4.55	2.147	36	77	
7	Control	236	224	−12	0.733	8.2	6.00	1.948	32	62	
8	26	270	312	+42	0.801	6.3	5.08	2.289	27	62	
9	Control	236	238	+2	0.654	5.9	3.87	2.107	33	70	
10	26	251	290	+39	0.713	7.0	5.02	2.259	29	66	
11	Control	248	240	−8	0.769	5.7	4.36	1.949	25	49	
12	15	250	280	+30	0.759	6.1	4.67	2.820	11	31	Tumors†
13	Control		164		0.427	9.2	3.92	1.187	41	49	
14	32		184		0.498	6.2	3.11	1.629	35	57	
15	Control		177		0.511	8.4	4.28	1.479	35	52	
16	32		214		0.623	6.4	4.02	1.841	37	68	

* These figures represent milligrams of inorganic phosphorus liberated per hour from a substrate of sodium-beta-glycerol phosphate by the enzyme in 25 cc. of tissue extract (from one gram of tissue) at a temperature of 38°C. and maintained at a pH of 8.8 by a glycine-NaOH buffer mixture.

† Nos. 5 and 12 had large tumors in abdominal cavity; probably ovarian cysts or infected uterus.

analytical balance, care being taken to avoid loss of moisture. The extraction and phosphatase determinations were carried out as described by Wilkins and Regen (3).

Of the 16 animals used, two proved to have abdominal tumors (5 and 12)

which made them unfit for the purpose of the experiment; however, as a matter of interest, phosphatase determinations were made on one of these animals (12). As shown in table 1 the injected animals gained from 30 to 67 grams while the controls gained 2 to 3 grams or lost 6 to 12 grams. The left femurs of the injected animals (with the exception of one animal with a tumor, 12) weighed from 2.3 to 22.0 per cent more than their respective controls. The kidneys of the injected animals weighed from 5.3 to 37.2 per cent more than those of the corresponding controls.

With two exceptions (10 and 12), the bones of the injected animals showed a slightly *lower* phosphatase activity per unit of weight than the corresponding controls. The total activity per bone showed more variation; however, it was not appreciably higher in any experimental bone than in its control. This was contrary to the expected findings since it was thought that the phosphatase activity of adult bones would be increased as a result of the renewed growth produced by the growth hormone.

Wilkins and Regen (3, 4) have found that the phosphatase activity of adult as well as growing bone is *decreased* following deep x-ray therapy. The activity of the enzyme may be *increased* in adult bone by certain means. It has been found in this and other laboratories (4, 5, 6, 7) that the enzyme is greatly increased following fracture. This tends to indicate that phosphatase plays a part in bone regeneration. Since phosphatase is thought to play an important part in the growth of young bone, it is not clear why it is not increased in these adult bones which were stimulated to new growth by the hormone. Does this suggest that the mechanism involved in this renewed growth of adult bone is different from that concerned in the growth of young bone?

The phosphatase activity of the kidneys showed the same trend as that of the bones. In each instance, with one exception (16), the phosphatase activity per unit of weight of the kidneys of the injected animals was somewhat lower than that of the corresponding controls. Whether this slight decrease was due directly or indirectly to the hormone, to the slight tissue damage caused by the injections, or to some other factor, we cannot say. It may be of interest to note that the kidneys of 12, one of the tumor animals, showed a very low phosphatase activity.

SUMMARY AND CONCLUSION

Injection of anterior pituitary growth hormone into adult rats was accompanied by an increase in body, bone and kidney weights. The bones and kidneys of the injected animals showed a slightly lower phosphatase activity per unit of weight than those of the corresponding controls. Thus it appears that renewed growth of adult rat bone is not accompanied by an increase in phosphatase activity.

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THE PRIMACY OF POLYURIA IN DIABETES INSIPIDUS

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It is well known that the two clinical symptoms characteristic of diabetes insipidus are polyuria and polydipsia. However, it is not known definitely which of the two is primary, whether the thirst is due to marked water loss by way of the kidney, or whether the increased urinary output follows as a natural physiological consequence of the thirst.

Inasmuch as a definite answer to this question seemed to be of considerable importance for the understanding of the mechanism involved in the production of the diabetes insipidus, it was decided to make a special study of the causal relationship between the polydipsia and polyuria.

The rat was chosen as the experimental animal because diabetes insipidus can be produced in it with fairly consistent success and because its water-intake and urinary output can be measured easily and accurately—advantages which make it possible to procure data from large numbers of animals.

The experiments were performed in two series. In one series urine measurements were made on animals with acute and chronic diabetes insipidus completely deprived of water to determine whether, in the absence of water, polyuria still persists. In the other series an attempt was made to determine which of the two cardinal symptoms, polyuria or polydipsia, appears first after the operation.

METHODS. The operative technique has been described in detail in previous papers (Richter, 1930 and 1934; Richter and Wislocki, 1930). It will be sufficient to state here that diabetes insipidus was produced either by a stab wound with a fine scalpel through the sphenoid bone near the anterior margin of the pituitary gland, or by total or partial removal of the gland by suction applied through a hole in the sphenoid bone.

Animals under observation were kept in small individual cages 7 inches long, $4\frac{1}{2}$ inches wide and $4\frac{1}{2}$ inches deep with a wire mesh bottom. The urine, falling through the mesh to an inclined trough below, was collected in graduated glass cylinders.

RESULTS. *Polyuria of rats with acute diabetes insipidus deprived of all water.* The thirteen rats with acute diabetes insipidus immediately after

operation excreted a large quantity of urine even though deprived of all water. Typical records of a hypophysectomized and a control animal are presented in figure 1 A. The control animal was exposed to the same operative procedure except for the removal of the hypophysis. The urine excretion differed strikingly both in the hourly and total output; the control excreted only 1.7 cc. in twelve hours whereas the experimental animal excreted 25.5 cc. or 8 per cent of its body weight.

The results of this group of experiments are summarized in table 1. The effect of the operative procedure when successfully performed is obvious.

Polyuria of rats with chronic diabetes insipidus deprived of all water. Similar results were obtained with rats with diabetes insipidus of long

TABLE 1

Urine in cubic centimeters excreted during nine hour period immediately following operation by animals deprived of all water

CONTROL RATS		RATS WITH ACUTE DIABETES INSIPIDUS	
Rat 1	1.8	Rat 6	12.7
2	3.3	7	12.1
3	2.5	8	16.6
4	1.7	9	16.5
5	3.2	10	19.4
		11	27.1
		12	19.3
		13	18.4
		14	25.5
		15	19.9
		16	18.4
		17	14.8
		18	24.4
Average.....	2.5		18.8

standing. In a typical record (fig. 1 B) from an animal in which diabetes insipidus had been established sixteen days previously, the output each hour for twelve hours was abnormally high with a total output of 23.8 cc. for the twelve-hour period. For purposes of comparison a typical record is presented of the urine excreted by a normal animal under the same conditions. It can be seen that the hourly output was very much lower than in the experimental animal, and the total output for twelve hours was 3 cc. as compared to 23.8 cc.

The results of these experiments are summarized in table 2 which gives the total urine output for one nine-hour period for twelve normal animals and four animals with diabetes insipidus. The output for the normals varied from 0.7 to 3.4 cc. with an average of 1.5 cc., while the output of the

animals with diabetes insipidus ranged from 4.7 to 23.8 cc. with an average of 10.2 cc. It will be noted further that the output of these animals varied considerably when measured at five to twenty-day intervals; the record for rat 14 of table 2, which is typical, shows readings of 9.0, 8.7, 11.1,

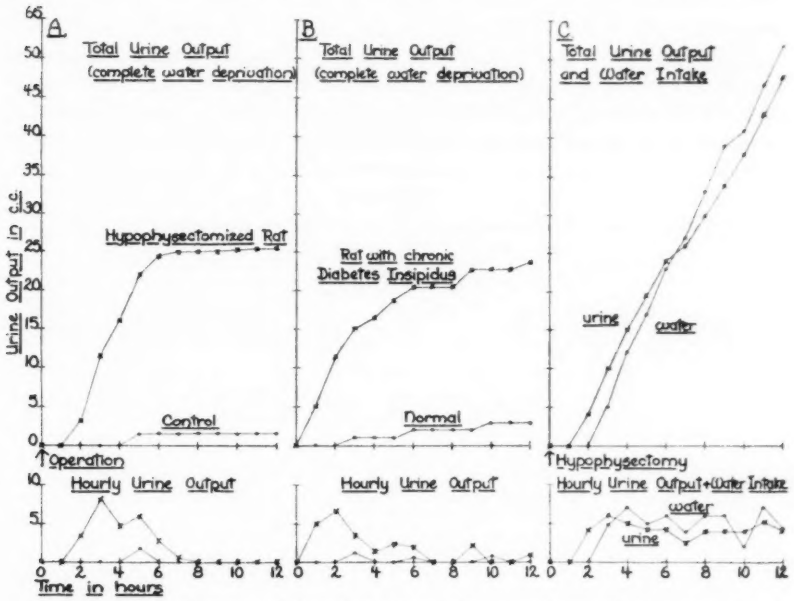

Fig. 1 A. Typical curves of the urine output of an experimental and a control rat, both deprived of water. The experimental animal was hypophysectomized, the control rat was subjected to a similar operation except for the removal of the gland. Urine output is indicated on the ordinates in cubic centimeters.

Upper chart: Total urine output for twelve-hour period.

Lower chart: Hourly urine output.

Fig. 1 B. Typical curves of the urine output of a rat with chronic diabetes insipidus, and a normal rat.

Fig. 1 C. Typical curves showing the onset of polyuria and polydipsia after hypophysectomy. Urine output and water intake are indicated on the ordinates in cubic centimeters.

Upper chart: Total urine output and water-intake for twelve-hour period.

Lower chart: Hourly urine output and water-intake.

and 18.3 cc. on four different days. The basis for these fluctuations is undetermined. They do not seem to be correlated with the degree of polydipsia present as shown in the last column.

It is apparent that animals with chronic as well as acute diabetes insipidus lose large amounts of urine when completely deprived of water.

The priority of the onset of polyuria following hypophysectomy. In these experiments seven hypophysectomized animals were given free access to water so that records were obtained of water-intake as well as urine output. It was found that the polyuria resulting from hypophysectomy preceded the polydipsia by intervals varying from 50 minutes to 1 hour and 50 minutes, with an average of 1 hour and 21 minutes (see table 3). A typical record from one of these animals is presented in figure 1 C in which urine output and water-intake in cubic centimeters are indicated on the ordinates and time in hours on the abscissae. The upper chart records the total amount of urine excreted and the total amount of water consumed during

TABLE 2

Urine output in cubic centimeters per nine hour period of animals deprived of all water

CONTROL RATS		RATS WITH CHRONIC DIABETES INSIPIDUS		
Rat no.	Total urine output per nine hour period	Rat no.	Total urine output per nine hour period	Post-operative average of daily water intake for ten days before intensive experiment
1	1.2	13	23.8	116.9
2	1.1	13	6.3	101.0
3	1.6	13	5.2	90.9
4	3.4	14	9.0	96.7
5	1.6	14	8.7	90.0
6	1.9	14	11.1	101.8
7	2.2	14	18.3	119.5
8	1.0	15	9.6	78.4
9	1.5	15	10.4	67.1
10	0.7	15	9.0	54.5
11	0.8	16	9.0	101.6
12	1.0	16	9.0	92.9
		16	4.7	113.2
		16	8.2	102.9
Average.....	1.5		10.2	94.8

the twelve hour period following operation; the lower chart shows the urine excretion and water-intake during each one-hour period. It will be noted that at the end of the second hour after hypophysectomy the urine output was 4.8 cc. while the water-intake was still zero. The polydipsia did not start until toward the end of the third hour, so that the polyuria preceded the polydipsia by 1 hour and 25 minutes.

Attention may be drawn here to the fact that, although the onset of polyuria definitely preceded the onset of the polydipsia, the water-intake overtook the urine output by the fourth hour after the hypophysectomy. After that a fairly constant difference of approximately 2 cc. was main-

tained between the water-intake and urine output, the former remaining higher, due partly to the loss of water through channels other than the urine tract such as the lungs and skin, and partly to the loss of urine by evaporation in the collecting troughs.

The results of these experiments support the view that the polyuria is the primary symptom in diabetes insipidus and that the thirst results from the consequent water loss.

Discussion. Observations on the problem of the primacy of polydipsia or polyuria have been made previously, incidental to other experiments on diabetes insipidus. The conflicting results of different workers, however, did not make it possible to draw any definite conclusions. Bailey and Bremer (1921) and Curtis (1924) reported that the polydipsia is primary, while Bourquin (1927) found the polyuria to be primary. The

TABLE 3

Time of onset of polyuria and polydipsia after hypophysectomy

RAT NO.	POLYURIA	POLYDIPSIA	PRECEDENCE OF POLYURIA
1	1 hr., 40 min.	2 hr., 50 min.	1 hr., 10 min.
2	2 hr., 30 min.	4 hr.	1 hr., 30 min.
3	1 hr., 25 min.	2 hr., 40 min.	1 hr., 15 min.
4	3 hr., 10 min.	4 hr., 30 min.	1 hr., 20 min.
5	4 hr., 5 min.	5 hr., 30 min.	1 hr., 25 min.
6	1 hr., 30 min.	3 hr., 20 min.	1 hr., 50 min.
7	4 hr., 25 min.	5 hr., 15 min.	50 min.
Average.....	2 hr., 41 min.	4 hr.	1 hr., 21 min.

results of the present experiments are in agreement with those reported by Bourquin.

The significance of these results as regards the etiology of diabetes insipidus may now be considered. The fact that the polyuria was found to be primary means that the structures injured by the hypophysectomy or stab wound in the hypophyseal region must have some control over the function of the kidney. So far as is known at the present time there are two structures in this region which are reported to participate in kidney regulation: 1, the hypothetical renal center in the hypothalamus; and 2, the posterior lobe (and intermediate lobe) of the hypophysis. All the evidence at hand indicates that nervous impulses from renal centers to the kidneys play little or no part in the regulation of kidney function (Bourquin; Theobald and Verney, 1935; Bailey and Bremer, etc.). There is, however, definite clinical as well as laboratory evidence that the posterior lobe does play an active and important part through the secretion of the antidiuretic pituitrin. (van de Velden, 1913; Gersh, 1934; Burgess, Har-

vey and Marshall, 1933; Cushing, 1933.) Removal, then, of the pituitrin, which normally inhibits kidney function, should cause a diuresis.

It may be noted, in agreement with Greving (1926), that the lesion necessary to produce this result may be located either in the posterior lobe itself (Richter, 1934); in the stalk which contains the fibres from the supra-optic nuclei (Cushing, 1932; Richter, 1933); or in the supra-optic nuclei themselves (Ingram, Fisher and Barris, 1934). The first eliminates the pituitrin at the source, the second and third eliminate it by taking away the stimuli which cause the posterior lobe to produce it.

The one obstacle to the full acceptance of this formulation lies in the fact that the complete removal of the pituitary gland does not always produce diabetes insipidus, and when it does the diabetes insipidus is never permanent. This means that some additional factor must be taken into consideration. In a previous paper it was reported that the anterior lobe plays an important part in the etiology of the syndrome, inasmuch as polyuria or polydipsia are never permanent unless anterior lobe tissue remains (Richter, 1934), possibly due to the presence of a diuretic substance in the anterior lobe. On this basis the diabetes insipidus can be accounted for in terms of a disturbance of the balance between the anti-diuretic pituitrin from the posterior lobe and the diuretic agent from the anterior lobe.

SUMMARY

1. Rats with acute diabetes insipidus produced by hypophysectomy or a stab wound in the floor of the third ventricle excreted large quantities of urine when deprived of water. The average for thirteen rats for a nine-hour period was 18.8 cc. as compared to 2.5 cc. for five rats with a control operation.

2. Rats with chronic diabetes insipidus were found to excrete large quantities of urine even when completely deprived of water. The average for four rats for a nine-hour period was 10.2 cc. as compared to 1.5 cc. for twelve normal rats.

3. The onset of polyuria preceded the onset of polydipsia by an average of 1 hour 21 minutes following the hypophysectomy in seven animals.

4. These experiments indicate that polyuria is the primary factor in the syndrome of diabetes insipidus.

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OBSERVATIONS ON THE LATE EFFECTS OF DENERVATION OF THE CAROTID SINUSES AND SECTION OF THE DEPRESSOR NERVES

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Certain of the functions assigned to the carotid sinus and aortic nerves by earlier workers have recently been subjected to critical restudy, chiefly because the original observations were based upon acute experiments and it seemed possible that the observations did not reflect the true functional value of these structures. Particularly in question has been the influence of the sinus and aortic nerves in the regulation of respiration.

In carrying out the studies described below an attempt was made to meet the objections that might be directed toward previous work, such as the non-physiological character of acute experiments, the neglect to denervate the cardio-aortic areas, and the practice of sectioning the depressor nerves in the neck, which in the dog is an uncertain procedure. Three dogs were available which had been completely denervated for 11, 10 and 1 month respectively. The operation consisted of denervation of the carotid sinuses, section of the right cardiac branches of the vagus by the transpleural route and section of the left vagus nerve in the neck. Employing these animals observations were made on the respiratory effects of carbon dioxide, the circulatory effects of amyl nitrite and asphyxia and on the presence of nerve regeneration.

METHODS. The first series of experiments were performed to compare the respiratory reactions of operated and of unoperated dogs to inhalation of increasing concentrations of carbon dioxide. The animals were made to breathe through a mask into a closed system of approximately fifty liters, capacity containing one part of oxygen and four parts of air. Circulation and thorough mixture of the gases was maintained by a rotary pump which was connected through a "Benedict-Roth" metabolism machine with a large reservoir. By means of the tracings made with this machine on a slowly moving kymograph the respiratory rate, amplitude, tidal volume and hence the total ventilation per half minute interval was determined. In making the tracings, the dogs were allowed to breathe for one-half to one minute from the air-oxygen mixture to produce a normal tracing and then

carbon dioxide was run into the system by displacing it from a container with water flowing at a constant rate from the reservoir of the system. By this means the total gas volume was kept constant. The observations were

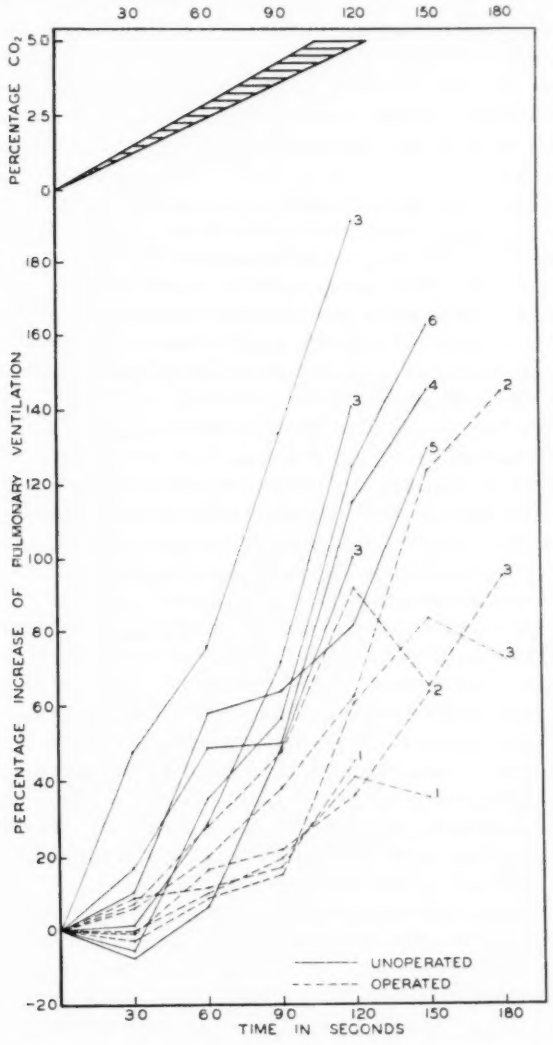

Fig. 1. Showing the percentage increase in pulmonary ventilation during inhalation of increasing concentrations of carbon dioxide. The zero point represents the tidal output per minute when the animal breathed air-oxygen mixture.

carried out at intervals over a period of two months and the most perfect of a large number of tracings were employed in the preparation of the graph.

Finally the animals were lightly anesthetized with ether and a blood pressure tracing taken from the femoral artery. The blood pressure responses to occlusion of both carotid arteries before and after section of the right vagus nerve, to amyl nitrite and to asphyxia were then recorded.

RESULTS. In figure 1 a delay in the response to carbon dioxide in the operated as compared to the unoperated animals is apparent. A comparison of the response in the same animal before and after operation is seen only in dog 3.

The response of the blood pressure in the operated animals to occlusion of the carotid arteries was negligible, there being only the slight mechanical elevation which occurs upon occluding the artery to any large vascular area. This was true both before and after section of the remaining vagus trunk. Section of this nerve had no significant effect upon the heart rate, the increases being respectively 26, 9 and 0 beats per minute in the three animals. Stimulation of the distal end of the right vagus with faradic current gave a normal cardio-inhibitory response.

Inhalation of amyl nitrite produced a fall in blood pressure which was qualitatively normal.

Asphyxia from clamping the trachea caused a moderate rise in blood pressure. The type of curve was practically identical in the three dogs, and the elevation was somewhat less marked than is usually seen following this procedure in animals with intact depressor mechanisms.

In the above experiments we have obtained our results under conditions not previously realized with absolute certainty. Our animals were essentially normal in all respects except that the carotid sinus and cardio-aortic depressor nerves were completely severed. Proof of the functional absence of these nerves was shown in the terminal experiments by the lack of response to occlusion of the common carotid arteries and by the failure of the hypertension and tachycardia to recur following vagotomy. It is an interesting fact that in spite of the extensive operation, some of the cardio-inhibitory fibers persisted. They are assumed to have reached the heart by leaving the vagus trunk with its pulmonary branches which, except for the first one or two, were carefully preserved.

The experiments of Cromer and Ivy (1931), of Gemmill et al. (1933) and of De Groat, Davis and McDonald (1933) seemed to show that under normal conditions denervation of the sinuses was without effect upon respiration. Since these reports appeared, the importance of the sinuses in the respiratory response to anoxemia has been convincingly demonstrated, but experiments with carbon dioxide have been conflicting. Schmidt (1932) reports that the response of anesthetized animals to carbon dioxide is unchanged after denervation of the sinuses. Selladurai and

Wright (1932), however, showed that anesthetized and decerebrate cats with sinuses denervated give a smaller response to inspired carbon dioxide than do unoperated animals. From our own results, which are in substantial agreement with those of Selladurai and Wright, it would appear that the sinuses do have a function in the respiratory response to carbon dioxide, particularly in the low concentrations but that this is not the major factor involved.

That the arterial hypertension which accompanies denervation of the vaso-sensory areas of the sinuses and of the aorta is relatively transient has already been demonstrated (De Groat, Davis and McDonald, 1933; E. Koch, 1934; Heymans and Bouckaert, 1934; Green, De Groat and McDonald, 1935). This result has been attributed to regeneration of the nerves (Koch and Mies, 1929), to the presence of depressor fibers in the vagus other than the main depressor bundle (Goormaghtigh, 1931) and to a lack of sympathetic tonus imposed by the conditions (complete rest) under which the blood pressure is determined (Heymans and Bouckaert, 1934). The functional absence of the carotid sinus and vagus depressor fibers in our animals demonstrates that the failure of the hypertension to maintain itself must be due to compensation by some other mechanism.

Our results do not confirm the statements that denervation of the sinuses reverses the effects of the nitrites on blood pressure (Dautreband, 1934) nor does asphyxia produce a fall in blood pressure. Although the rise in pressure which occurred in our animals was uniformly less than is usually seen, because of the small number of animals and the normal variations which occur in this type of experiment, we are unable to state whether or not there is an altered response.

CONCLUSIONS

1. In three dogs with the sinus and aortic depressor nerves destroyed for periods of 1, 10 and 11 months respectively, a reduction in the degree of respiratory response to carbon dioxide was observed.

2. The failure of the hypertension which follows denervation to be maintained is the result of adaptation rather than regeneration of the carotid sinus and aortic depressor nerves.

3. Destruction of the depressor system is without qualitative effect upon the blood pressure response to amyl nitrite and to asphyxia.

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THE EFFECT OF HYPOPHYSECTOMY AND CEREBRAL MANIPULATION IN THE DOG UPON THE RESPONSE OF THE BLOOD SUGAR AND INORGANIC PHOSPHORUS TO INSULIN[1]

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It has long been recognized that the hypophysis can to some degree control metabolic processes, particularly that of carbohydrates (1, 2, 3). The most striking demonstration of a change in the carbohydrate metabolism of the hypophysectomized animal was found in its abnormal sensitivity to insulin, an observation first made by Houssay and Magenta in 1924 (4), by Geiling, Campbell and Ishikawa in 1927 (5), and since then by many others (6–11). The attempts, however, to determine which part of the gland influences carbohydrate metabolism have yielded conflicting results. The small size of the hypophysis and its inaccessibility render difficult the removal of one lobe without interference with its neighboring lobe or parts. Moreover, because of their proximity to the gland, injury to the tuber cinereum, infundibulum, and hypothalamus may be readily associated with hypophysectomy. It is by no means unlikely that the failure to assess carefully the true damage to the various tissues at the base of the brain accounts, in part at least, for the conflicting views regarding which one of the lobes, when injured or excised, is associated with permanent changes in the carbohydrate metabolism of the organism.

In the course of a study of the metabolism of the hypophysectomized dog, it was deemed advisable to provide so-called "operated controls," i.e., dogs in which all cerebral manipulations up to, but not including, excision of the gland itself were carried out. A study of this preparation as well as of the hypophysectomized animal seemed necessary since the intracranial approach for the removal of the gland involves interference with neighboring tissues in order to bring the gland into full view. It was found that insulin sensitivity could occur in animals in which the hypophysis had been exposed to inspection but still left entirely intact. A study was therefore

[1] The expense of this investigation was defrayed in part by a grant to one of us (I. L. C.) from the Research Board of the University of California, Berkeley.

made of the relation of cranial and cerebral manipulation to insulin sensitivity.

EXPERIMENTAL. Full-grown bitches were used in this investigation. With the exception of those designated by N, which were normal, all dogs were subjected to cranial and cerebral manipulations that varied in degree from an incision of the dura to complete excision of the hypophysis. The animals have been grouped according to the operative procedure to which they were subjected.

TABLE 1

The effect of insulin on blood sugar and inorganic phosphorus of normal dogs

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive, and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
NI. On diet Oct. 30, 1933	Dec. 10	9.5	86.9	80.9	20	3.75	3.34	50
	Feb. 10	10.7	84.5	78.0	110	4.00	3.55	50
	Mar. 3	11.0	83.3	83.3	30	3.52	3.52	30
	May 6	11.8	82.1	77.4	165	3.69	3.39	60
NII. On diet Nov. 15, 1933	Dec. 20	11.2	85.7	76.2	20	3.16	2.67	50
	May 12	13.8	82.1	78.0	105	3.47	3.29	30
NIII. On diet Nov. 22, 1933	Dec. 22	11.2	92.8	88.1	60	3.34	3.07	125
	Feb. 22	12.6	85.7	80.9	20	3.40	3.20	20
	Apr. 21	13.2	75.0	66.7	20			
NIV. On diet Nov. 22, 1933	Feb. 17	12.0	75.0	71.4	80	4.00	3.70	80
	May 9	13.4	73.8	69.7	55			

* Postabsorptive value.

† Minutes after insulin injection.

1. *Craniotomy, retraction of the right temporal lobe, and hypophysectomy* (table 2). The details of this operation have been previously described (12). After the brain had been shrunk by means of an intravenous administration of a concentrated solution of sodium chloride (0.33 gm. per kilo of body weight in a 20 per cent solution), the right temporal muscle was incised parallel to its origin and stripped from the skull. After cranial and dural openings had been made in the area from which the muscle had been removed, the animal was turned on its side, the head rotated so that the vertex was down, the right temporal lobe retracted by means of a spatula

and the hypophyseal region exposed to view. The hypophysis was then removed and the stalk cauterized. *Retraction upon the temporal lobe was*

TABLE 2

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy, cerebral retraction and hypophysectomy had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
HI. Operated on Nov. 5, 1933	Dec. 10	11.5	84.5	44.0	95	3.46	1.67	95
	Dec. 22	12.0	75.0	41.7	120	3.07	2.10	185
	Feb. 10	13.6	85.7	47.6	110	2.70	1.82	210
	May 6	15.5	75.0	31.5	60	3.07	1.59	165
HII. Operated on Nov. 10, 1933	Dec. 17	7.5	92.8	52.4	110	4.14	3.43	110
	Feb. 17	8.7	88.1	79.8	60	4.00	4.00	
	May 12	9.8	88.1	79.7	115	4.28	3.06	65
HIII. Operated on Nov. 8, 1933	Dec. 20	9.0	92.8	47.6	50	4.44	2.92	180
	Feb. 17	10.5	78.6	41.0	110	3.80	2.86	175
	May 6	13.0	70.2	57.1	105	3.34	3.00	165
HIV. Operated on Dec. 27, 1933	Feb. 22	10.2	69.0	35.7	105			
	Apr. 21	12.5	80.9	46.9	105	3.08	2.45	105
HV. Operated on Dec. 27, 1933	Feb. 22	8.2	85.7	52.4	100	3.40	3.00	160
	Apr. 21	10.0	83.3	42.9	160	4.90	3.58	100
HVI. Operated on Jan. 3, 1934	Mar. 3‡	7.5	42.9	19.0	60	3.52	2.65	60
	May 9§	7.5	38.7	20.2	60	4.00	2.70	60
HVII. Operated on Jan. 3, 1934	Mar. 3	6.9	71.4	35.7	105	2.86	1.75	105
	May 9	7.7	59.5	29.7	180	3.29	2.22	180

* Postabsorptive value.

† Minutes after insulin injection.

‡ Convulsions occurred 75 minutes after insulin injection.

§ Convulsions occurred 85 minutes after insulin injection. This animal ate only one-half of its last meal on the day prior to experiment.

never maintained longer than 2 minutes. Retraction of the temporal lobe for longer than 3 or 4 minutes was found to result in profound postoperative disturbances such as drowsiness, walking in circles, weakness of the left

side, and convulsions. Even death from cerebral edema and increased intracranial pressure may result from too long retraction during this stage of the operation. In the application of the cautery to the base of the brain, care was taken to avoid tissue other than the stalk. The whole procedure from time of incision of the skin to closure of the wound *never occupied more than 20 minutes.* Seven such dogs are recorded in table 2.

In all dogs of this group, a study was made at necropsy of serial sections, 10μ thick, of the whole hypophyseal region. There was a complete ab-

TABLE 3

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy and cerebral retraction had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
CI. Operated on Dec. 4, 1933	Dec. 17	9.0	84.5	54.8	50	3.24	2.79	50
	Dec. 22	9.0	85.7	59.5	50	3.53	2.79	20
	Feb. 10	10.8	83.3	71.4	120	2.67	2.35	120
	May 6	13.3	72.6	51.8	20	2.73	2.19	50
CII. Operated on Dec. 4, 1933	Dec. 20	7.6	80.9	71.4	110	3.53	3.16	50
	Feb. 17	8.7	79.8	75.5	110	3.00	3.00	
	May 9	9.5	72.6	71.4	20	3.00	2.76	20
CIII. Operated on Jan. 3, 1934	Mar. 3	9.5	75.5	50.0	155	3.52	2.90	50
	May 12	12.0	86.9	75.0	70	3.74	3.70	
CIV. Operated on Jan. 25, 1934	Feb. 22	9.8	88.1	50.0	60	3.30	2.80	100
	Apr. 21	10.5	85.7	45.8	160	2.67	1.85	100

* Postabsorptive value.

† Minutes after insulin injection.

sence of anterior, posterior, and intermediate lobes of the gland as well as of all stalk tissue. Small pieces of the pars tuberalis, the largest measuring 1.6 × 2.0 × 0.5 mm., were present at the floor of the third ventricle in dogs HI, HII, HIII and HIV. In the other animals no pars-tuberalis cells were found.

2. *Craniotomy and cerebral retraction* (table 3). In this group of 4 dogs all the operative manipulations up to, but not including, the removal of the hypophysis were carried out. The dogs received the intravenous injection

of the hypertonic solution of sodium chloride. Craniotomy was performed immediately beneath the right temporal muscle after it had been partly freed of its attachment to the skull and the brain exposed by an incision through the dura. A spatula was then inserted and the right temporal lobe of the brain retracted so that the hypophysis and base of the brain were brought into view. *The retraction was maintained for one minute or less* and the wound closed in the usual manner. The whole procedure at no time occupied more than 20 minutes. Thus there was no mechanical interference with the hypophysis during the course of the operation. Inasmuch as hypophysectomy by the intracranial route involves extensive manipulation of the brain before the gland becomes visible for its removal, the dogs in this second group serve as control preparations for the hypophysectomized animals described above.

The brains of these dogs were carefully examined at necropsy for cortical and hypophyseal damage. In addition, serial sections, 10μ thick, were made of the whole hypophyseal region and the condition of the gland carefully studied. A variable degree of adherence between muscle and meninges of the parietal lobe was present at the site of the craniotomy. There was a slight compression of the right lateral ventricle in the 4 dogs of this group but otherwise the brain appeared normal. The microscopic study of the pituitary gland revealed that this structure was completely intact and normal.

3. *Craniotomy and opening of the dura.* The 3 animals recorded in table 4 received the intravenous injection of sodium chloride. Craniotomy was performed beneath the right temporal muscle and the bone removed. The dura was then radially incised; the brain and arachnoid, however, were not disturbed. The wound was then closed in the usual manner.

4. *Normal dogs.* The 4 animals shown in table 1 suffered no operative interferences with cranium or brain.

During their entire stay in the laboratory, the dogs in the 4 groups were maintained under identical environmental and dietary conditions. Each animal received twice daily, at 8:00 a.m. and at 4:00 p.m., a mixture containing 125 grams of lean meat, 40 grams of sucrose and 5 grams of bone ash. In addition, the vitamins were added to this diet twice weekly, vitamin B in the form of a rice-bran concentrate, and vitamins A and D in the form of cod liver oil. With the exception of the first week following the operation, during which time the appetites of these animals were somewhat poor, all dogs, with few exceptions, possessed good appetites, ingesting all food soon after it was served.

In order to compare their sensitivity to insulin, the dogs were injected subcutaneously with 1/16 unit of the hormone per kilo of body weight, the fall in blood sugar and inorganic phosphorus being used as the index of the animal's response to insulin. The insulin was diluted with 0.9 per cent

sterile salt solution so that the amount to be injected was always contained in 1.0 cc. This dose was adopted, since in a study of the effects of small doses of insulin on the blood sugar of the rabbit, Scott and Dotti (13) found doses smaller than 1/16 unit impractical because of the lack of sufficient precision in blood sugar methods. These workers have also shown that, as judged by the fall in blood sugar, the effects of small doses of insulin in normal animals of varying weights are strictly comparable when the dose is measured in units per kilo of body weight. Thus the minimum dose, the effect of which is experimentally demonstrable in normal animals, has been employed in the present investigation.

The dogs were in the postabsorptive state at the time the insulin was injected, having ingested their last meal 16 hours prior to the removal of the first sample of blood. Blood was taken from the femoral arteries. Eight samples of 5 cc. each were removed from each dog during the course of an experiment. Since the dogs had been previously accustomed to the procedure, most of them showed little, if any, struggle during the removal of blood samples.

Blood sugar was determined with the copper-iodometric reagent of Shaffer and Somogyi (14), while the filtrate was obtained by the precipitation of blood with zinc hydroxide (15). For the determination of inorganic phosphorus Tisdall's method (16) was employed. Whole blood was added, with vigorous shaking, to 5 volumes of 10 per cent trichloracetic acid and immediately thereafter the mixture was rapidly filtered. As soon as a sufficient volume of filtrate was observed in the flask, 5 cc. were transferred to a 15-cc. centrifuge tube and the inorganic phosphate immediately precipitated with strychnine molybdate. Following the washing and centrifugation of the precipitate, the supernatant fluid was decanted and the tube set aside until the other blood samples had been similarly treated. The filtration and isolation of the inorganic phosphorus of the blood, when carried out with sufficient rapidity, avoid the interference that may result from the hydrolysis of a labile phosphoric ester. All determinations were made in duplicate and the results recorded represent the means of closely agreeing values.

Results. *Normal dogs* (table 1). The postabsorptive values for blood sugar, observed over a period of 5 to 6 months, ranged between 73.8 and 92.8 mgm. per cent, whereas those for inorganic phosphorus lay between 3.16 and 4.00 mgm. In a single experiment no fall in blood sugar followed the injection of 1/16 unit of insulin per kilo, whereas during the course of the 10 other tests the lowest blood sugar values were 5 to 11 per cent below preinjection values. The maximum reduction in blood inorganic phosphorus produced by the same dose of the hormone in 9 experiments was 15 per cent.

Dogs in which craniotomy, retraction of the right temporal lobe, and com-

plete hypophysectomy had been performed. The observations recorded in table 2 were made on 7 dogs over a period of 4 to 6 months following complete hypophysectomy. The postabsorptive blood sugar values in dogs HI–HV ranged between 69.0 and 92.8 mgm. per cent and the inorganic phosphorus between 2.70 and 4.90 mgm. per cent. The lowest blood sugar values were found in dogs HVI and HVII. In the former the 2 values observed were 42.9 and 38.7, whereas in the latter they were 71.4 and 59.5 mgm. per cent.

In HI, which was tested for insulin sensitivity on 4 different occasions, the minimum values found for blood sugar and inorganic phosphorus were

TABLE 4

The effect of insulin on blood sugar and inorganic phosphorus of dogs in which craniotomy and radial incision of the dura had been performed

Insulin dose in all cases 1/16 unit per kilo of body weight. Two values only are recorded, (1) postabsorptive and (2) minimum observed following the insulin injection.

DOG	DATE OF EXPERIMENT	WEIGHT, KGM.	BLOOD SUGAR			BLOOD INORGANIC PHOSPHORUS		
			Preinjection,* mgm. per cent	Minimum		Preinjection,* mgm. per cent	Minimum	
				Mgm. per cent	Time,† min.		Mgm. per cent	Time,† min.
RI. Operated on July 12, 1934	Sept. 8	13.5	90.5	80.9	60	4.25	3.75	60
RII. Operated on July 12, 1934	Sept. 8	11.5	76.2	65.5	105	3.77	3.49	60
RIII. Operated on July 12, 1934	Sept. 8	12.5	78.6	67.8	100	3.68	3.39	60

* Postabsorptive value.

† Minutes after insulin injection.

respectively 44 to 58 and 32 to 51 per cent below initial values. The most pronounced insulin response in HII occurred in the first experiment after hypophysectomy, at which time the blood sugar showed a maximum reduction of 44 per cent. In the 2 later tests, performed at about 3 and 6 months after the operation, the lowest blood sugar values recorded were 9.5 per cent below the postabsorptive values. In HIII, reductions in blood sugar to the extent of 49, 48, and 19 per cent followed the injection of the hormone. HIV and HV, which were tested for insulin sensitivity at 2 months and again at 4 months after hypophysectomy, showed maximum falls of 36 to 48 per cent in blood sugar. The administration of 1/16 unit of insulin per kilo resulted in convulsions in HVI, while in HVII the

blood sugar fell 36 and 50 per cent on the 2 occasions when its insulin reactions were followed.

Dogs in which craniotomy and retraction of the right temporal lobe had been performed (table 3). In the 4 dogs of this group the postabsorptive values for blood sugar ranged between 72.6 and 88.1 mgm. per cent and for inorganic phosphorus between 2.67 and 3.74 mgm. per cent. CII received insulin injections on 3 occasions and at these times the response to the hormone was no greater than that observed in normal dogs, the maximum fall being to the extent of 12 per cent of the initial value. The 3 other dogs were definitely more sensitive to insulin than the normals. Thus in the 4 experiments carried out on CI over a period of 5 months, the maximum falls in blood sugar were 35, 31, 14 and 29 per cent. Two months after the operation CIII showed a maximum drop of 34 per cent in blood sugar, while 10 weeks later the same dose led to a maximum fall of 14 per cent which, however, was maintained for at least 165 minutes. In the 2 experiments performed on CIV about 1 and 3 months after the operation, the blood sugar was reduced by 43 and 46 per cent respectively.

Dogs in which craniotomy and radial incision of the dura had been performed (table 4). In these animals the blood sugar and inorganic phosphorus were found to be normal, as regards both their postabsorptive values and their reactions to the subcutaneous injection of 1/16 unit of insulin per kilo.

Inasmuch as the 4 groups of dogs received the same diet throughout their stay in the laboratory, variations in their insulin sensitivity cannot be ascribed to differences in caloric ingestion. All dogs were in good nutritional state, and, except for HII and HVII, possessed good appetites, immediately ingesting all food served. HII took several hours to ingest a meal completely, while HVII sometimes failed to consume the whole of one meal by the time the next was served.

DISCUSSION AND SUMMARY

The intracranial approach for the removal of the hypophysis involves 3 distinct manipulations: 1, craniotomy and incision of the dura mater; 2, retraction of the right temporal lobe, and 3, interference in the region of the base of the brain, this last being a procedure that consists in the excision of the gland and in the application of cautery to the stalk. In the present investigation the insulin sensitivity of 3 groups of dogs, in which the various stages had been performed, was compared with the normal. Craniotomy and radial incision of the dura did not alter the sensitivity of dogs to insulin. On the other hand, animals in which all 3 manipulations had been performed, i.e., a complete hypophysectomy, showed a greatly increased sensitivity to the hormone. A dose of insulin, namely, 1/16 unit per kilo, that in normal dogs led to average maximum drops of 5 to 8 per cent in blood sugar and 7 to 10 per cent in blood inorganic phosphorus produced

in the 7 completely hypophysectomized dogs average maximum reductions of 21 to 52 per cent in blood sugar and 16 to 41 per cent in inorganic phosphorus. Although in 2 of these dogs, namely, HII and HIII, the later responses were not as severe as those observed about 1 month after the operation, nevertheless the fact that the same degree of sensitivity was found in HI at 1 month and also at 6 months after the hypophysectomy strongly suggests that the exaggerated insulin reaction is a permanent characteristic of these dogs.

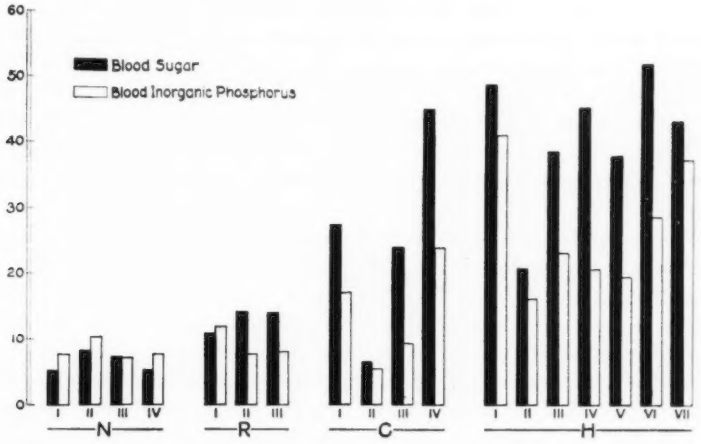

Chart 1. The insulin sensitivity of normal and operated dogs. The scale represents the average of the maximum percentage reductions in blood sugar and inorganic phosphorus produced by the subcutaneous injection of 1/16 unit of insulin per kilo of body weight.

N—normal dogs. *R*—dogs in which craniotomy and radial incision of the dura had been performed. *C*—dogs in which craniotomy, incision of the dura and retraction of the right temporal lobe had been performed. *H*—dogs in which craniotomy, incision of the dura, retraction of the right temporal lobe and complete hypophysectomy had been performed.

Despite the fact that subsequent microscopic study of serial sections of the whole hypophyseal region of all 4 dogs that were subjected to the first 2 manipulations, namely craniotomy and retraction of the right temporal lobe, revealed normal hypophyses, the insulin reaction of only one of these (CII) can be considered entirely normal (chart 1). It is indeed striking that at 3 months after a cranial operation involving no *apparent* mechanical interference with the hypophysis the injection of 1/16 unit of insulin per kilo should have resulted in a drop in blood sugar to the extent of 46 per cent and in inorganic phosphorus of 31 per cent (CIV).

The augmented sensitivity to insulin produced in dogs by craniotomy

and retraction must be the result of the latter manipulation, for dogs in which craniotomy and radial incision of the dura had been performed responded to insulin in a normal manner. Since retraction of the right temporal lobe was involved in the operative removal of the hypophysis, it is necessary to consider the relation of the insulin sensitivity induced by craniotomy and temporal lobe retraction to the sensitivity found after complete hypophysectomy. Chart 1 clearly shows that the degree of sensitivity in the former case was not always as great nor as uniform as in the latter. It seems a reasonable inference, therefore, that part of the increased insulin reaction observed in the dogs hypophysectomized by the intracranial route may be the result of the retraction. On the other hand, although it is not possible in the case of each hypophysectomized dog to determine the extent of the sensitivity conferred by retraction on one of the lobes, it is nevertheless evident that interference in the hypophyseal region cannot be excluded as the major factor in the causation of insulin sensitivity.

The mechanism whereby operative manipulation of the cerebral hemisphere is capable of producing a variable, but nonetheless significant, increase in the degree of sensitivity to insulin is not known at present. As judged by gross and histological examination of the base of the brain, the hypophyses of all dogs subjected to craniotomy and temporal lobe retraction were intact and normal. Some attachment between the meninges of the parietal lobe of the brain and the muscle at the site of the craniotomy had occurred, but it seems unlikely that this could have caused a greater sensitivity to insulin since similar attachments were found after simple craniotomy. Although the cortex of the brain appeared normal and showed normal staining qualities, the right lateral ventricles (operated side) of these 4 dogs were found slightly compressed. It is difficult to relate compression of the ventricles to insulin sensitivity. The fact that insulin sensitivity was most pronounced in dogs that had suffered the greatest damage to the hypophyseal region (excision of the whole gland and cautery of its stalk) suggests that the augmented sensitivity observed in animals after craniotomy and retraction of the temporal lobe was also caused by some interference in this region. Although the pituitary gland itself was not touched, it should be recalled that in this group of dogs retraction was carried as far as the sella turcica so that the gland was exposed to full view. In so doing, it is not unlikely that one or more structures in the region of the hypophysis were injured. The large number of structures, with as yet poorly understood functions, that are present in the small area around the pituitary gland makes it difficult to avoid injury to one tissue while attempting to affect another. Hence identification of the structure or structures responsible for insulin sensitivity can be at present only a matter for speculation.

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EXPERIMENTS ON THE HYPOTHALAMIC NUCLEI IN THE REGULATION OF CHLORIDE AND SUGAR METABOLISM

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The fact that lesions of the hypothalamus lead to hyperglycemia and glycosuria and further to polyuria with hyperchloremia was shown by the experiments of Aschner (1912), Camus and Roussy (1922), Leschke (1919), Bailey and Bremer (1921), etc. The question, however, which structures or nuclei of the hypothalamus had to be involved to produce these two effects remained unclarified. It was open to discussion whether the increase of blood sugar was not merely the consequence of handling and tying up of the animal, of narcosis and shock, as Tannenbaum and Hiller (1929) assumed.

The first suggestion of an identification of a hypothalamic cell group connected with the sugar metabolism was made by Brugsch, Dresel and Lewy (1920) when they found that piqûre into the dorsal autonomous nucleus of the 10th nerve—followed by glycosuria—led to a retrograde degeneration in the periventricular nucleus. This name was adopted by F. H. Lewy for a cell group situated around the fornix bundle within the hypothalamus. This nucleus was found to be degenerated in a peculiar way in Parkinson's disease in which an alimentary hyperglycemia is mostly present (Dresel and Lewy, 1922).

The hypochloruric polyuria with hyperchloremia seemed to be connected with the paroptic ganglion (Gudden) since F. H. Lewy (1922) in two cases of diabetes insipidus without a pituitary lesion found a marked lesion of this nucleus. The subsequent experimental destruction of the pars posterior of the pituitary body was found to be followed by a retrograde degeneration of the cells of the paroptic ganglion (Kary, 1924).

In order to corroborate these two hypotheses, experiments were made on 33 cats in which either the periventricular nucleus or the paroptic ganglion or both were unilaterally stimulated and subsequently destroyed with the help of the Clarke-Horsley apparatus. Serial histological sections

[1] The experiments were initiated in the Laboratory of the 2nd Medical Clinic of the Charité, Berlin.

through the operated brains showed that in 12 animals the periventricular nucleus, in 6 the paroptic ganglion, in 4 both nuclei were destroyed; 11 animals in which the lesion did not involve either of the two nuclei served as controls.

The publication of our results had to be postponed for a couple of years owing to recent events. In the meantime, the close relationship between the periventricular nucleus and the sugar metabolism has been proved by Loyal Davis, Cleveland and Ingram (1934). We shall, therefore, only refer briefly to this problem.

Penfield's (1934) objections to Davis' experiments are not valid in our case as we were able to approach the nuclei by means of the spherical model of the Clarke apparatus from all planes and sides.

The following protocols illustrate each group of experiments.

TABLE 1

DAY	URINE	SPECIFIC GRAVITY	NaCl	NaCl	BLOOD CHLORIDE	NON PROTEIN UREA
	cc.		*grams per cent*	*grams per day*	*mgm. per cent*	*mgm. per cent*
1	80	1009	0.72	0.57	602	24
2	70	1015	0.92	0.64	575	
3	85	1012	0.59	0.47	607	
			Operation			
4	40	1042	0.198	0.079		
5					635	72
6	15	1025	0.55	0.082		
7	40	1022	1.3*	0.52	634	

* Eight hours after load with 1.7 gram NaCl in 90 cc. water.

Cat 10. Two thousand three hundred grams. The cat was kept on a constant diet for 8 days and the quantity of output of fluid and NaCl was followed. Pernocton 1 cc. intravenously. Needle inserted laterally on right side and local damage produced. *Next morning* the posterior extremities were spastic, but the animal was active and took fluid and food spontaneously. *Second day* after operation animal was completely anuric. *Third day*: animal drank satisfactorily. Urine normal. *Fourth day* after operation: animal tested by increased NaCl and water load (1.7 gram NaCl in 50 cc. water).

Figure 1 shows the NaCl and sugar level in the blood following the unilateral stimulation and destruction of the paroptic ganglion. Table 1 gives the water and Cl balance in the urine before and after operation, fig. 2 the blood chloride curve with a load of 1.7 gram NaCl before and after operation.

The curves show that the immediate effect of the unilateral stimulation and destruction of the paroptic ganglion is a rise in the blood NaCl from 580 to 670 mgm. per cent lasting longer than 9 hours and levelling finally between 624 and 634 mgm. per cent, i.e., considerably higher than before the operation. There is a hyperchloremia.

Finally, the blood chloride tolerance curve after load with 1.7 gram NaCl is en-

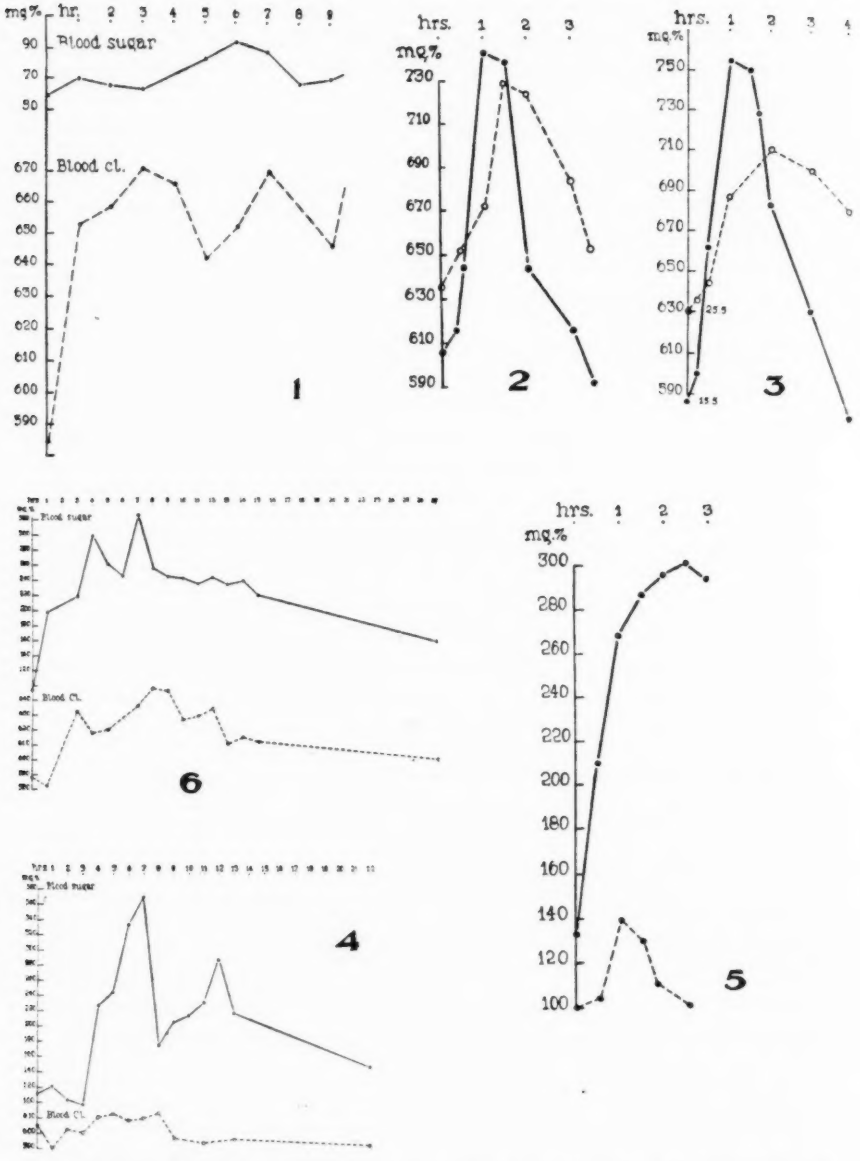

Fig. 1. Cat 10. Blood sugar and blood chlorides after unilateral stimulation and destruction of the paroptic ganglion. Blood sugar not influenced. Increase of the blood chlorides.

tirely different before and after operation. After the operation the curve rises and falls more slowly and the total increase is smaller. The curve, therefore, is not so high and not so steep as in normal conditions.

As this is the most striking point of the present investigations we add in table 2 and figure 3 the chloride-water balance and the tolerance curve of another animal (cat 13) before and after stimulation and destruction of one paroptic ganglion. The figures show the same behavior as in table 1 and figure 2 and the high specific gravity is proof enough that the quantity of urine is credible and that the urine was not lost.

The *lesion* in cat 10 extended over about 1 mm. The lesion was in front of the brain stem next to the ascending fornix column around the chiasm and destroyed the ganglion paropticum. The lesion was nowhere in the neighbourhood of the periventricular nucleus.

To give a clear idea of the entirely different effect of the unilateral stimulation and destruction of the periventricular nucleus we point to figures 4 and 5.

Cat 5. One thousand, nine hundred and fifty grams. Pernocton 1 cc. intravenously. Needle inserted from left lateral aspect and somewhat from above. Destruction with 20 mA 15 seconds.

In the *blood sugar curve* (fig. 4) a maximal increase occurred after an elapse of 7 hours with a second summit 12 hours after operation. In this cat the blood chlorides were normal throughout the whole time of observation. Figure 5 represents the result of a load with 7 grams glucose before and after the operation.

The *lesion* extended over about 1.5 mm. The lesion was in the posterior part of the diencephalon at the superior border of the hypothalamus and covered the periventricular nucleus.

Lastly, cat 9 is an example of a combined lesion of paroptic and periventricular nuclei.

Cat 9. Two thousand, five hundred and fifteen grams. One cubic centimeter pernocton intravenously. Needle inserted nearly horizontally from the left side. The *next day* the cat was sitting, the hind limbs in spastic contraction. The animal in

Fig. 2. Cat 10. Blood chloride tolerance curves with an extra load of 1.7 grams NaCl, before and after unilateral destruction of the paroptic ganglion. Full line before, dotted line after operation.

Fig. 3. Cat 13. Blood chloride tolerance curves with an extra load of 1.7 grams NaCl, before (full line) and after unilateral destruction of the paroptic ganglion (dotted line).

Fig. 4. Cat 5. Blood sugar and blood chlorides after unilateral stimulation and destruction of the periventricular (perifornical) nucleus. Blood chlorides not influenced. Blood sugar increase with a peak at the 7th and at the 12th hour respectively after operation.

Fig. 5. Cat 5. Blood sugar tolerance curve with an extra load of 7 grams glucose, before (dotted line) and after unilateral destruction of the periventricular nucleus (full line).

Fig. 6. Cat 9. Blood sugar and blood chloride curves after unilateral stimulation and destruction of the paroptic ganglion with secondary softening of the hypothalamus involving the periventricular nucleus.

trying to walk fell to the left side. The *second day* the animal kept a compulsory posture on its left side. As we did not succeed in feeding the cat, it was sacrificed.

Examination showed sugar and chlorides increased in the blood 3 hours after operation coming back to normal very slowly in the following 20 hours (fig. 6).

The needle penetrated laterally and reached the paroptic ganglion around the optic tract. The direct lesion was accompanied by a secondary softening of the ipsilateral hypothalamus, pushing the third ventricule to the opposite side and involving the periventricular nucleus.

Discussion. 1. Blood sugar curves as represented in figure 1 are not a rarity. They show that catching and handling of the animals and even electrical stimulation and destruction of an area distant from the periventricular nucleus do not increase blood sugar.

2. Comparison of figures 1 and 4 shows that blood sugar and blood chlorides can vary independently as a consequence of lesions of two distinct cell groups in different regions of the hypothalamus. *A lesion of the paroptic ganglion increases the blood chlorides and not the blood sugar, a lesion of the periventricular nucleus increases the blood sugar and not the blood chlorides.* It is evident that an electrical stimulus can never be exactly limited to a minute area, but the effect is less according to the distance of the lesion from the nucleus.

We can, therefore, entirely corroborate the investigations of Davis, Cleveland and Ingram, although our modus procedendi was somewhat different.

3. There is a great difference between the increase of blood sugar and chlorides following *a*, a piqûre of the dorsal vagus nucleus and the reticular substance; *b*, of the cerebellar vermis and *c*, one into the hypothalamic nuclei. Both the form and temporal pattern of the curves differ widely with the place of stimulation.

One may say that the characteristic of *blood chloride and blood sugar curves after lesions of the medulla or the cerebeller vermis* is a quick increase and a gradual decrease within a few hours (Lewy and Shinosaki, 1926; Shinosaki, 1926).

Following a *unilateral lesion of the hypothalamus* the curves look entirely different. The blood chlorides reach the summit of the curve not before the third, often only in the sixth hour after operation. These values are maintained for from 9 to 12 hours after operation or may decrease for a while to increase a second time at the 8th to 10th hour. The normal, as a rule, is not reached before the next day and the level may remain above the normal for a number of days.

Still more characteristic is the delayed type of curve with two distinct peaks in the blood sugar. Here the first maximum is reached, on the average, within 4 to 7 hours. Then follows a marked decrease, although not to the normal and at the 7th to 12th hours after operation a second elevation follows which mostly is not so high as the first. Not until the

day after the operation does the sugar level come back to the normal. Some curves were obtained which showed a rapid initial rise of the blood sugar and successive return to the normal, but we found always that with such curves the seat of the lesion was not within the periventricular nucleus.

Neither the blood chloride nor the blood sugar curves have the smooth decrease which is characteristic for the oblongata, the cerebellar, the adrenalin curves and the curve after a load. Although the general trend is downward there occur irregularities which seem connected in some way with the food intake.

4. The curves with *chloride and sugar load after unilateral hypothalamic lesion* start after the lesion from a higher level, increase and decrease much more slowly. They reach the summit in 2 to 3 hours instead of in 1 hour and are in 3 hours, as a rule, not yet back to the normal. A certain difference is seen in the degree of reaction in that the added chlorides disappear

TABLE 2

DAY	URINE	OUTPUT			INTAKE			BLOOD CHLORIDE
		Specific gravity	NaCl	NaCl	Milk	Meat	Bread	
	cc.		*grams per cent*	*grams per day*	*cc.*	*grams*	*grams*	*mgm. per cent*
1	90	1023	0.55	0.49	200	50	50	583
2	120	1022	0.50	0.60	200	50	50	
3	90	1015	0.53	0.48	200	50	50	585
				Operation				
4	25	1052	0.13	0.33				
5	25	1046	0.14	0.034	100	25		
6	30	1048	0.19	0.051	200	50	25	631

after operation more quickly from the blood than in normal conditions while the sugar is changed into glycogen and deposited in the liver more slowly than normally. Thus the sugar curve appears much higher, the chloride curve lower than before the operation but the general form is similar in both of them. The conception of an opposite direction in the time of output of chlorides and sugar after lesion of the central nuclei and tracts is strengthend by the observation of Tokay (1931).

The most important point of our investigations—in our opinion—is the *relation of the chlorides in the blood to the output in the urine and* the question *of water* output after lesion of the paroptic ganglion. Our results are entirely different from those after the salt-water piqûre of Jungmann and Meyer (1913) into the oblongata and from those after unilateral striatum lesions (Tokay, 1931). Veil (1930) considered the derangement of the *salt balance to be hyperchloremic—hypochloruric in unilateral lesions of the hypothalamic centers*; our experiments confirm this.

SUMMARY

1. Unilateral stimulation and subsequent destruction of the paroptic ganglion (Gudden) with help of the sterotaxic apparatus is followed by an increase of the blood chlorides. The chlorides reach the summit of the curve in the 3rd to 6th hour and sometimes attain a second maximum in the 8th to 10th hour after operation. An elevation of the blood chlorides may be present for several days.

The output of chlorides and of urine is distinctly decreased in percentage and in absolute figures.

The blood chloride curve with a load of 1.7 grams NaCl rises and drops considerably more slowly after a lesion of the hypothalamus.

This lesion had no influence upon the blood sugar level.

2. Unilateral stimulation and subsequent destruction of the (perifornical) periventricular nucleus is followed by an increase of the blood sugar. The blood sugar curve reaches its peak at the 4th to 7th hour. Then it drops and has its second maximum within the 7th to 12th hour after operation. The curve returns to the normal from one to three days after operation.

The blood sugar tolerance curve with a load of 7 grams glucose rises and drops considerably more slowly after lesion of the hypothalamus.

This lesion has no influence upon the blood chloride level.

3. Only lesions involving these nuclei have maximal effects on the blood chlorides and the blood sugar.

4. It is open to discussion whether the effect is the consequence of a stimulation or of a temporary paralysis.

5. The impairment of the salt metabolism in hypothalamic lesions is characterized as hyperchloremic—hypochloruric.

6. As these experiments were unilateral and acute they can be directly compared in their results neither with bilateral destruction in animals nor with the chronic human disease of diabetes insipidus.

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ON THE EXISTENCE OF PRO-SECRETIN

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In 1902 Bayliss and Starling published their original researches on the hormone control of the external secretion of the pancreas. They found that acid solutions would extract secretin from the mucosa of dog duodena while neutral solutions would not affect the extraction, at least not nearly so well. For that reason they postulated the existence of a precursor, prosecretin, which was hydrolyzed by acids to yield active secretin.

Since that time there have been many investigations on the secretin problem. In the main, however, they have been directed toward the proof of the physiological part played by secretin and toward the isolation of the hormone. A much smaller number have been concerned with the question of the existence of prosecretin. For that reason many of the published opinions concerning the prohormone have been made from incidental observations, rather than from directed experiments planned to give information concerning its existence or properties. It is only in recent years that the proof of the rôle of secretin in the digestive process has been accepted by the majority of the workers in the field, and it is therefore not surprising that this particular problem has been relatively neglected.

The literature on the subject has been recently reviewed by Still (1931) and although a bibliography is appended to this paper, a review of the literature will not be included here.

In this study we have reopened the question and have attempted to obtain concrete evidence concerning the existence of prosecretin.

EXPERIMENTAL. In order that the work in all the experiments reported in this paper would be comparable, all were of one general plan. Except in special instances, all extracts of the mucosa were made in the ratio 1:20 with respect to the particular solvent used. The insoluble residues were removed by centrifugation, the extracts were neutralized and 5 cc. portions were injected intravenously into dogs prepared for secretin assay in the manner described in the review by Still (1931). In any given experiment, then, a fairly accurate comparison of the activity of the various extracts was obtained and a less accurate comparison of the activity of extracts used in different experiments. A description of the methods

used in preparing the extracts and special points concerning some of the experiments, will be included in the proper place in the text of the paper.

The experiments were planned with the hope of preparing, and possibly purifying, an extract of the duodenal mucosa which would possess certain properties, namely, that the intravenous injection of it would cause no secretion of pancreatic juice until acidified or that the secretagogue potency already present would be greatly increased by acidification.

Series I. Experiments on "untreated mucosa." In these experiments, fresh mucosa was thoroughly ground with sand and extracted with the solvent used. The mixture was centrifuged and the filtrate neutralized, when necessary. The neutralized filtrate was assayed in 5 cc. quantities.

Dog mucosa. A. Fresh dog mucosa was extracted with 0.9 per cent sodium chloride solution, in the manner described above and the extract was divided into two portions "a" and "b." Extract "a" was injected without change or additions to it. Extract "b" was strongly acidified with HCl and after standing for a few minutes an equivalent amount was

TABLE 1

The effect of acidification of neutral saline extracts of fresh duodenal mucosa of the dog

EXTRACT	TREATMENT OF THE MUCOSA	PANCREATIC RESPONSE
		cc.
"a"	Neutral saline extract	0.3
"b"	Neutral extract—acidified	0.2
"c"	Acid extract of the residue	2.2

neutralized and injected for assay. The residue, from the above process, was extracted with 0.4 per cent HCl and centrifuged; the filtrate "c" was neutralized and injected for assay. Table 1 shows the results of one typical experiment.

By reëxtracting fresh dog mucosa with neutral saline from four to ten times, the quantity of secretin which could be extracted with saline in succeeding extracts rapidly diminished to sub-threshold quantities. The residue, however, upon acid extraction yielded abundant secretin.

When the cellular continuity of the mucosa was destroyed by repeated freezing with CO_2 snow and ether, the results were similar to those already noted except in a few instances in which the saline extract "b" developed slightly more activity upon acidification than was found in the saline extract "a." This can be considered only as mildly suggestive evidence for the existence of prosecretin because the increase was small and such preparations all contained large amounts of noncentrifugable material. Furthermore, the extracts made from the "frozen" mucosa were extremely toxic and it was felt that data from them should be used only as confirmatory evidence.

Similar experiments were carried out in which "frozen" mucosa was dropped into boiling 0.4 per cent HCl or 0.9 per cent NaCl solutions, then cooled and centrifuged. In this way the coagulable protein was removed. The secretin content of these filtrates was found to be in the approximate ratio of 20:1.

Hog mucosa. B. Experiments similar to those described under "A" were conducted, using fresh hog mucosa. The results were essentially the same as the above experiments employing fresh dog mucosa, except for one feature. The amount of saline extractable secretin was more in every instance than that found in the case of dog mucosa. In fact, it was found frequently that almost as much saline extractable as acid extractable secretin was present in the fresh hog mucosa. This difference is not due to differences in freshness of the mucosa and may be due to a species difference.

These results are similar to those of Bayliss and Starling since they show that prosecretin is not soluble in neutral saline. In addition, these experiments show the following: 1. A portion of the secretin is present in the mucosa in an "active" form and may be extracted with neutral saline. The quantity of this saline extractable secretin is variable from animal to animal and there is apparently a greater difference in the relative amounts in different species of animals. 2. After repeated extractions of the "fresh untreated" mucosa with saline, further saline extracts of this mucosa do not contain a threshold quantity of secretin. The residue, however, will yield a considerable quantity of secretin to acid extraction.

It should also be mentioned that reagents other than acids may extract secretin from the fresh mucosa which has been repeatedly washed with saline as described above; such solutions as dilute alkali and aqueous alcohol belong to this group, the alcohol being much more efficient than the dilute alkali.

The preparations just described in this series have contained large quantities of vasodilatin, together with other toxic substances. Attempts were made to "salt-out," with neutral salts, a relatively non-toxic precipitate from slightly alkaline saline suspensions of fresh mucosa. It was hoped that the precipitate would contain not only secretin but its precursor, prosecretin. These experiments were unsuccessful in the latter respect because all of the secretin present in the precipitate could be removed by neutral saline and acidification of the saline extract did not enhance its secretagogue action. Experiments along this line were discontinued.

Series II. Experiments on dried mucosa. The question was raised during the progress of the experiments of series I, concerning the ability of saline to penetrate the cells of the mucosa rapidly enough to extract the secretin. The following experiments were planned with that point in mind. It should be mentioned that Stepp (1912) was probably the first to work

with dried mucosa. His product contained secretin which could be extracted by saline, acids, bile, glycerine and aqueous alcohol. This finding led him to suggest several kinds of secretin.

A. *The "dried mucosa."* Preparations were made by the following procedure. Fresh mucosa of the dog was spread in a thin layer on glass plates and dried in a current of warm air. The dried scales were then milled to pass through a 400 mesh wire sieve.

This preparation yielded only a small amount of secretin on extraction with saline, dilute alkali, and bile salts. However, aqueous alcohol and acids extracted considerable secretin from the dried preparation, the latter extractant being the most efficient. The relative amounts of secretin extracted from the mucosa which had been dried, by these solvents, was of the order obtained from the "untreated fresh mucosa" (series I) treated with the same solvents. It was discovered that the preparation developed considerable acidity during the drying process. The need of a dried preparation which remained neutral or slightly alkaline, was obvious.

B. *"Dried-heated-mucosa."* In order to eliminate as much as possible the development of acidity, the preparation was made in a manner similar to the above (A. "Dried mucosa") except for a preliminary heat treatment of 30 minutes' duration at 80°C., to reduce the effects of enzymes and bacteria.

This product was similar to A ("dried-mucosa") except that more secretin, which was soluble in saline, was present in this mucosa.

C. *"Dried-heated-alkaline-mucosa."* To further eliminate the possible effects of acid development, preparations were made which were kept slightly alkaline (to phenolphthalein) but otherwise prepared as the "heated-dried-mucosa" (B).

This preparation reacted to solvents in a manner similar to the above mentioned preparation.

It should be noted with respect to these preparations that heating causes more of the secretin to become saline soluble. Table 2 shows the results of an experiment in which aliquot samples of the same mucosa were used in making of "dried-mucosa" (A), and "dried-heated-mucosa" (B), of both hog and dog mucosa. The table also indicates the larger amount of saline soluble secretin in the saline extracts.

D. *"Alkaline-crude."* If one extracts the "dried-heated-alkaline-mucosa" (C) repeatedly with saline, the saline soluble secretin can be removed. The residue may be dried with acetone and ether and preserved as a dry powder which possesses the following properties: a. The preparation will yield secretin activity to: 1, dilute mineral acids; 2, stronger organic acids; 3, seventy per cent aqueous alcohol; 4, hot neutral saline. b. The preparation will not yield secretin activity to: 1, NaCl solutions up to 5 per cent; 2, NaOH solutions up to 0.5 per cent; 3, sodium carbonate, 0.2 per cent; 4, solutions of bile salts.

Discussion of series I and II. These experiments show conclusively that the secretin activity of fresh mucosa is not all in the same state, because it is possible, using fresh mucosa (series I) or treated mucosa (series II), to wash out the saline soluble fraction so that further saline extraction fails to yield any activity. However, subsequent extraction of the residue with acids yields a considerable amount of secretin.

Although the experiments in series II are subject to the criticism that proteins of the mucosa have been denatured and possibly otherwise altered by reagents, the experiments of series I are not subject to that criticism.

These results might be interpreted in several ways, among which are: 1. The saline insoluble fraction represents prosecretin. 2. The saline insoluble fraction represents active secretin adsorbed on some of the colloids. 3. The saline soluble fraction represents prosecretin which has been activated by digestive acids but has not been absorbed into the blood stream.

Since we were unable to test the first interpretation and were able to examine the second and third, experiments were planned to that end.

TABLE 2

PREPARATION	ANIMAL	TREATMENT OF MUCOSA	PANCREATIC RESPONSE
			cc.
A	Dog	Unheated	0.2
B	Dog	Heated	1.5
A	Hog	Unheated	1.6
B	Hog	Heated	2.1

Series III. The adsorption of secretin. Recognizing the fact that the product "alkaline crude" (D) may have been denatured in the making, it was, however, selected for these experiments because it contained no saline soluble secretin and further saline extraction would not materially alter its composition. We have assumed the latter to be also the case of fresh, washed mucosa.

Two kinds of experiments were carried out to test the possibility of adsorption.

The secretin in the "alkaline crude" mucosa was inactivated with trypsin. The trypsin was destroyed by heating and the residue tested to show the absence of acid soluble secretin. Purified secretin was then added and the pH adjusted to approximately eight. After stirring for fifteen minutes the solid material was separated by centrifugation and the residue washed repeatedly with neutral saline. The residue was then dried with acetone and ether. The results showed that the powder yielded no secretin to extraction with saline but considerable secretin to acid extraction.

Eight experiments were performed in the following manner: One gram

of the "alkaline crude" preparation was extracted with 20 cc. of 0.4 per cent HCl and the filtrate divided into two portions, "a" and "b." Extract "a" was neutralized and 5 cc. injected intravenously for assay; extract "b" was made slightly alkaline (to phenolphthalein) and allowed to stand at room temperature for 15 minutes before injection. These extracts were prepared to demonstrate the quantity of secretin which the preparation would yield to acid extraction and to determine the effect of weakly alkalinizing such a filtrate. One gram of the "alkaline crude" preparation was then extracted with 20 cc. of 0.4 per cent HCl, the pH of the mixture was adjusted to about eight with NaOH and then stirred for 15 minutes. The mixture was then centrifuged and 5 cc. of the filtrate (just alkaline) constituted extract "c." Extract "d" was a 0.4 per cent HCl extract of the residue from "c." Extract "c" was prepared to determine if the adjustment of the pH results in adsorption of secretin. "d" was prepared to determine if the secretin which was adsorbed in "c" and

TABLE 3

Experiment showing the adsorption of secretin on activity "free" mucosa

EXTRACT	NATURE OF EXTRACT	PANCREATIC RESPONSE
		cc.
"a"	Control on "alkaline-crude" preparation to acid extraction	3.2
"b"	Effect of alkali on acid filtrate from the "alkaline-crude" preparation	2.6
"c"	Adsorption of secretin on activity "free" mucosa	0.0
"d"	Acid extract from the residue of "c"	2.8

which was not extractable with neutral saline, could be eluted with an acid solution. Typical results are shown in table 3.

These experiments indicate that in "heated-dried-washed-mucosa" preparations, the residual secretin which is soluble in acids may be adsorbed on the mucosa. This does not prove that secretin is so held in the living mucosa but only indicates that the treated mucosa can adsorb secretin at the proper pH. We are aware of the fact that the previous history of the mucosa may influence considerably such processes.

Series IV. The effect of the exclusion of digestive acids on the state of secretin in the mucosa. Thiry-Vella fistulae were made in ten dogs. The fistulae included the lower duodenum and upper jejunum. The animals were kept for six months or longer during which time the fistulae were frequently flushed with neutral saline. Thus the fistulae did not come in contact with free acids other than those which were formed during the metabolism of the tissues. At the end of the period the animals were sacri-

ficed and the fistulae immediately removed. The mucosa was scraped off and aliquot portions used to prepare extracts for secretin assay. These extracts compared at that time with extracts similarly prepared from normal dog mucosa.

The results were quite typical of those obtained with other fresh mucosae. The solutions which were effective in extracting secretin were, in order of increasing efficiency: saline, dilute alkali, aqueous alcohol and acids. These experiments strongly indicate that the saline extractable fraction of secretin, obtained previously, is not present due to the effect of digestive acids.

SUMMARY

It has been shown that the secretin in "untreated mucosa" exists in two forms with respect to the ability of solutions to extract it, one fraction which is readily extractable with neutral saline, the other which is extractable with acids and is not extractable with saline.

The saline extractable fraction might be termed "free" secretin and the fraction not extractable with saline called "bound" secretin. The "bound" secretin may also be extracted to a lesser degree by weak bases and aqueous alcohol.

We are not in a position to say anything concerning the condition in which "bound" secretin is found in the mucosa. However, we believe the "acid-hydrolysis" hypothesis of Bayliss and Starling not to be entirely tenable, inasmuch as there are many reagents other than acids, such as dilute alkali, aqueous alcohol, etc., which will extract the secretin. Our results only indicate that the "bound" secretin may be held by adsorption to the colloids of the mucosa.

It is not at all improbable that the "bound" secretin is combined with or adsorbed on protein molecules. The reagents which effectively extract it do so by hydrolysis or elution. In that respect "pro-secretin" probably does exist.

Whether or not the 'free" and liberated "bound" secretin are the same substance is undetermined.

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THE INFLUENCE OF HYPERPNEA AND OF VARIATIONS OF O_2- AND CO_2-TENSION IN THE INSPIRED AIR UPON HEARING

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It is generally known that in no other branch of physiological research is it more difficult to apply the results obtained on animals to human physiology than it is in observations concerning the brain. For this reason attempts have been made in recent times to perform experiments on anthropoids. However, even in these animals the study of sensory functions and their dependence on certain parts of the brain is extremely difficult as soon as a finer analysis is desired, because sensory disturbances become evident to the experimenter only in an indirect way through alterations in the motor behavior. For these reasons it seems desirable to study the functions of the central nervous system in the human, as far as circumstances permit.

The natural way of approach is, of course, that of a study of the functions of the sense organs under conditions which influence the excitability of the central nervous system. By making use of a spinal irradiation induced by peripheral stimuli some deeper insight into spinal physiological processes has been gained in previous experiments dealing with cutaneous sensations (Gellhorn, 1931–33). The present series of investigations concerns an alteration of the excitability of the cortex in humans and the effects brought about on various sensations, which were studied quantitatively. The factors to be investigated in this paper are variations in the O_2- CO_2-tension in the respiratory air, and thus indirectly in the blood and tissues. They were chosen not only because of the generally recognized importance of O_2 and CO_2 for the excitability of any living substance in general and of the nervous system in particular but also because of the rapidity of the gas exchange which restores normal conditions in the blood almost immediately. One of these factors (O_2-lack) has been frequently investigated for theoretical and practical reasons (aviation). However, even here a quantitative study in regard to sensations is only in the beginning. The relevant papers will be discussed later.

METHOD. The hearing experiments were conducted in a nearly sound-

proof room with a 2A Western Electric audiometer with which the threshold for hearing can be determined for eight different frequencies. The intensity is expressed in sensation units in percentages of the average hearing threshold for normal individuals. Ninety-six experiments were performed on six subjects who, after having been trained for several weeks, gave an almost absolute constancy of the hearing threshold. The procedure was as follows: The subject was connected with an audiometer and just prior to the release of the sound, a signal was given to start. Then followed sounds lasting from one to two seconds each in irregular intervals, starting with a sound twenty per cent stronger than that corresponding to the threshold and then gradually decreasing its intensity until the threshold was reached. The latter is defined by the correct response of the subject in three successive trials. The determinations were made in intervals of from 1 and 5 minutes since it had been found that readings taken in 1-minute intervals may cause fatigue particularly in experiments which extend over more than 40 to 60 minutes. The gas mixtures were prepared in several Douglas bags. The gas analysis was made with a Newcomer-Haldane apparatus.

RESULTS. *Oxygen-lack.* The first group of experiments is concerned with the influence of O_2-lack on hearing. Thirty-three experiments were made with O_2 concentrations varying from 7.5 to 15.8 per cent. The duration of the O_2-lack period varied between 8 and 30 minutes.

Oxygen-air mixtures of from 9 to 12 per cent O_2 showed a very marked effect. There was a gradual decrease in hearing, which began more or less rapidly, depending on the individual sensitivity of the experimental subject, the O_2-concentration used, and the duration of the O_2-lack period. In some cases the effects were immediately reversible after readmission of air; in others the hearing acuity remained diminished for various lengths of time.

Two experiments performed on the same experimental subject on different days are reproduced in figure 1. In both experiments the same concentration of oxygen was inhaled (10.4 per cent), but the duration of the O_2-lack period varied considerably. With the increasing duration the effect on hearing was intensified and the recovery delayed. In contrast to these findings it must be stated that the respiration returned to normal almost immediately after the readministration of air.

A series of experiments was then conducted to determine whether the slow recovery observed after a long period of O_2-lack could be hastened by the administration of 50 to 60 per cent O_2. The experiment reproduced in table 1 shows that breathing of such an O_2-rich mixture for more than 10 minutes does not appreciably alter the hearing threshold, a fact which, as will be shown later, seems to point definitely to a prolonged cellular change as a result of O_2-lack.

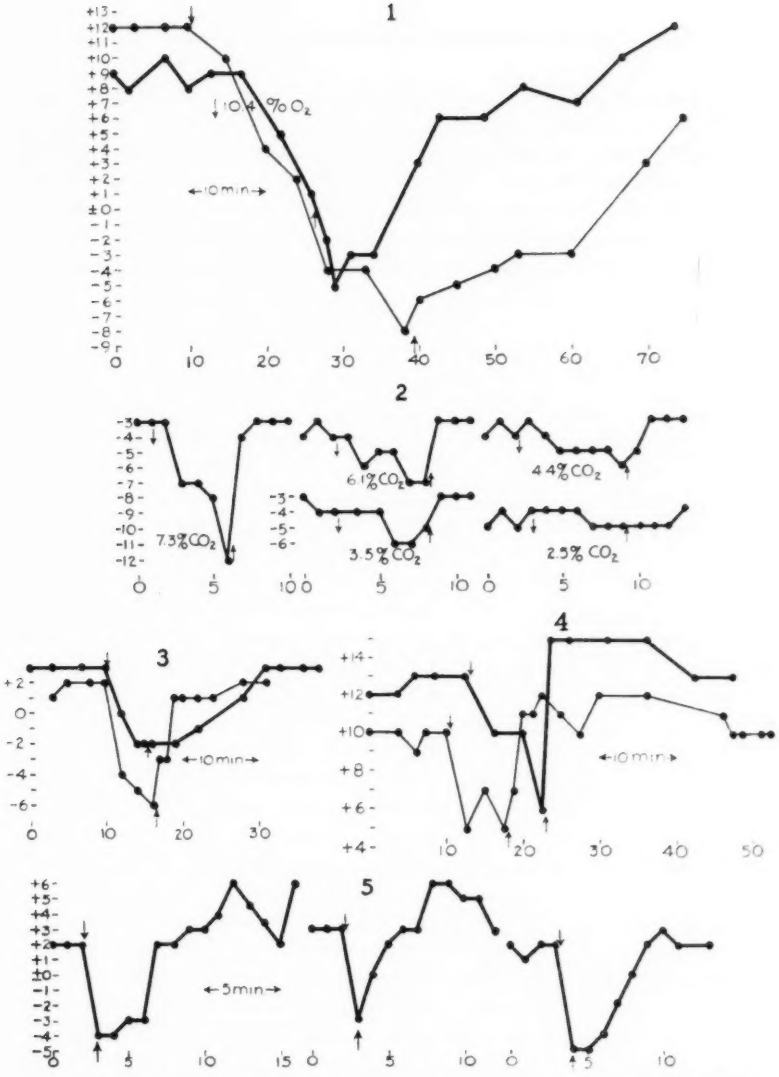

Fig. 1. In all figures: ordinate = auditory threshold in sensation units in percentage of the average human threshold for hearing. Abscissa = time in minutes. Between the arrows administration of N_2-air mixtures: In both experiments 10.4 per cent O_2.

Fig. 2. Between the arrows CO_2 as indicated on the graph. All experiments were done on the same person.

Fig. 3. Heavy line: 5.6 per cent CO_2. Thin line: 6.3 per cent CO_2.

Fig. 4. Heavy line: 7.05 per cent CO_2. Thin line: 6.9 per cent CO_2.

Fig. 5. Three experiments with hyperpnea (between the arrows) of 2 minutes each.

Oxygen in a concentration of 12 to 15 per cent inhaled for about 10 to 20 minutes sometimes produced a slight increase in hearing during the first few minutes, followed by a gradual decrease. The effects were slight but quite distinct, in view of the great constancy of the controls obtained before and after the O_2-lack period. They were readily reversible upon readministration of air. That we are here concerned with the effect of O_2-lack and not with other factors introduced by the breathing of a gas

TABLE 1

I. SUBJ.: ST. 5/21/34. SOUND: C1024

Time	Audiometer reading
A. Control period (air)	
minutes	
0	+8
2	+8
3	+9
6	+8
7	+9
B. Inhalation of 10.0 per cent O_2 for 30 minutes	
8	+6
14	+3
18	+3
24	+1
28	−2
30	−3
C. Control period (air) after O_2-lack	
2	−2
6	−1
11	−3
21	−2
29	+2
D. Inhalation of 50 per cent O_2 for 17 minutes	
minutes	
11	+3!!
17	+6
E. Control period (air)	
7	+6
12	+6
17	+4
74	+7
75	+8
77	+7

from a Douglas bag was shown by the control experiments in which the threshold was not altered when ordinary air was inhaled.

The typical course in all experiments described so far was that during the period of O_2-lack hearing was gradually decreased and was restored by the inhalation of air with a rapidity depending on the concentration of O_2 and the duration of the O_2-lack period. It must, however, be mentioned that in some of our experiments an improvement in hearing above the ordinary threshold was noticed during the first minutes when air was again inhaled. The improvement in hearing did not amount to more than

two or three points ordinarily, but in a few experiments quite excessive improvements lasting for several minutes were observed.

Experiments with CO_2. Thirty-seven experiments with CO_2 varying from 2 to 8.4 per cent (5–22 minutes' duration of exposure) were performed. Figure 2 shows the records of five experiments performed on the same individual with varying CO_2 concentrations. It is evident that 2.5 per cent CO_2 is without effect on the hearing threshold and that the first slight effect is seen with 3.5 per cent CO_2. With increasing concentration the hearing becomes progressively less. The experiments with other subjects gave very similar results, the threshold being around 4 per cent for an experiment lasting on an average about 6 to 10 minutes. Obviously, the mechanism involved in hearing is far less sensitive to CO_2 than the respiratory mechanism, since the breathing of 2.5 per cent CO_2 shows considerable change in the pneumographic record. Figure 3 shows that in CO_2 concentrations of about 6 per cent the decrease in hearing may persist for several minutes after readmission of air but it may be said that the recovery was in all experiments considerably faster than in experiments with O_2 of about 10 per cent, and of similar duration. Figure 4 illustrates not only the decrease in hearing resulting from the inhalation of 7 per cent CO_2 but also a very considerable improvement in hearing immediately after the readministration of air. As can be seen from the graphs, this improvement lasts over a considerable length of time and is then gradually replaced by the ordinary hearing threshold.

Hyperpnea and hearing. Twenty-six experiments were performed in which the influence of hyperpnea on hearing was studied. The experimental subjects inhaled as deeply as possible for 3 to 6 minutes according to the rhythm of a metronome. In some experiments the frequency was 35 per minute, in others considerably higher, up to 90 per minute. The results were consistent throughout the whole series of experiments and showed that immediately following the period of hyperpnea hearing was greatly decreased. Readings were not taken during the period of hyperpnea because of the impossibility of concentrating and breathing violently at the same time. Figure 5 shows several typical experiments. It indicates that a decrease in hearing may last as long as 10 minutes after such a period of hyperpnea. Therefore, it is present not only during the apnea but also at a time at which breathing has become perfectly normal. Whereas the third curve in figure 5 may be taken as a representative of experiments which showed a decrease in hearing after hyperpnea and a gradual recovery afterwards to a normal hearing threshold, the other two experiments indicate that after the threshold has become normal it may be followed by a period of hyperacusis.

Most experiments were carried out with C 1024 because we found a greater constancy of the hearing threshold for this than for any other tone.

However, when tones between C 128 and C 4096 were used similar results were obtained.

DISCUSSION. Summarizing these experiments, it may be said that under conditions of O_2-lack, CO_2-excess, and CO_2-lack, hearing is gradually decreased depending on the duration of these periods and the degree of change involved. After restoration of normal conditions hearing returns to the normal threshold. In some cases prior to this a period of hyperacusis may be observed in all three conditions. The changes induced by CO_2 are more readily reversible than those with O_2-lack and hyperpnea. In experiments with O_2-lack changes in threshold (decrease in hearing) were observed as long as two hours after the experiment.

The question arises as to the nature of the decrease in hearing observed in all three groups of experiments. There is a possibility that diminished hearing is due not to a diminished excitability of the fundamental processes involved in hearing but to an inability of the experimental subject to "concentrate the attention." Unfortunately, we do not know what attention is nor what physiological processes are called into play when we direct our attention to a sensation and perception. We only know that this peculiar process makes the sensations more vivid and distinct. It is, therefore, at least theoretically possible that a decrease in "attention" gives results similar to those obtained when the excitability is decreased. We believe, however, that this interpretation of our results is ruled out for the following reasons: First, the long training of the experimental subjects which made it highly improbable that the diminution in hearing was obtained because they were unable to concentrate their attention on the experiment; second, it was found that not infrequently at the end of the O_2-lack or hyperpnea period a period of improved hearing was obtained. This certainly could not have been observed with a diminished attention; third, measurement of attention (Bourdon test), to be reported elsewhere, showed only slight disturbances in attention which never persisted after air had been readmitted.

Instead of assuming that alterations in attention are responsible for the effects on hearing, an assumption which is highly improbable because of the great constancy of the controls before and after the experiment, it seems more reasonable to interpret the variations as being due to changes in the nervous mechanism involved in the sensation itself. Such an interpretation seems still more justified since our observations on the effect of O_2-lack are paralleled by Schubert's observations (1934) on the motor effect of O_2-lack in animals. He found that under the influence of a decreased oxygen tension in the air animals showed a decreased motor excitability leading to coma. After readmission of air an increased motor excitability was observed, leading even to convulsions. In view of these facts, we believe that the effects of O_2-lack and variations in the CO_2 tension lead to alterations in the excitability of the fundamental nervous mechanism.

We may then ask: Are these changes due to alterations in the circulation, or are they of cellular origin? It is well known that the inhalation of CO_2 and hyperpnea results in changes in blood pressure. We found, however, that in experiments on healthy, young individuals changes in blood pressure during and following the exposure to O_2-lack and CO_2-excess, as well as during hyperpnea, were extremely small. The greatest changes were observed with CO_2 and amounted to about 10 mm. mercury (systolic blood pressure). The decrease in blood pressure during hyperpnea was practically negligible and not outside of the experimental error. These findings agree well with the measurements of Voit (1933) and Raab (1929). That small changes in blood pressure are not responsible for the effects on hearing is borne out by experiments in which a considerably greater increase in blood pressure was obtained after physical exercise (20–30 mm. Hg) without influencing the hearing threshold. Furthermore, it must be emphasized that not infrequently long after-effects on hearing were observed after hyperpnea and O_2-lack, although the blood pressure remained completely unchanged.

Whereas a change in blood pressure can be eliminated as a cause of the effects on hearing observed in our experiments, the vasomotor reactions due to changes in the O_2- and CO_2-tension of the blood require a more detailed consideration. Schmidt and Pierson (1934) and Schmidt (1934) have shown that these factors alter the blood supply of the medulla oblongata and of the hypothalamus. Furthermore, direct observations of the pial vessels by Cobb and Fremont-Smith (1931), Wolff and Lennox (1930) and Lennox and Gibbs (1932) lead to similar results. It must, therefore, be assumed that both O_2-lack and CO_2-excess produce vasodilatation and increased blood flow, whereas the reduction in CO_2-tension in hyperpnea is accompanied by a reduction in the blood supply of the brain. The reduction in hearing during O_2-lack occurs, of course, not because of the increased blood supply but in spite of it. Obviously the increased blood supply cannot fully compensate for the diminished O_2-tension and as a result, the O_2-supply of the brain is reduced, although to a lesser extent than was to be expected without the vasomotor adjustment. We, therefore, obtain a diminished excitability, particularly of the cortical elements involved in hearing, since they are more sensitive to O_2-lack than any other elements of the central nervous system (concerning the literature compare Gildea and Cobb, 1930).

The very marked persistence of the effects of O_2-lack on hearing is well in line with the observations of Gildea and Cobb following a temporary complete anoxia of the brain, and seems to point to the ganglion cells of the cortex as the site of the changes. This assumption is supported by histological studies of these authors, who found the most pronounced O_2-lack effects in lamina III and IV of the cortex. As far as our own

observations are concerned, the fact that the effects of O_2-lack on hearing are independent of the frequency of the sound used seems in the light of experiments of Pearcy (1929) to favor such an interpretation. So do those experiments in which 50 to 60 per cent O_2 was administered after a period of O_2-lack. The fact that even after a 5 or 10 minute period of inhalation of 50 per cent oxygen no appreciable change in threshold occurred, seems to indicate a cellular change which persists in spite of the complete oxygenation of the blood.

The decrease in hearing obtained under the influence of CO_2-excess is in agreement with other effects of CO_2 on the central nervous system. King, Garrey and Bryan (1932) have shown that the knee jerk is decreased in the intact animal. Obviously CO_2 acts simply on the basis of its acid properties since Fröhlich and Solé (1924) have found that by perfusion of the spinal cord with acid solutions reflex activity completely disappears.

The explanation of the reduction in hearing observed after hyperpnea seems to be more complex. We must here distinguish the effects of increased alkalinity on blood vessels and on neurons. The latter seem to show increased excitability due to increased conductivity (Broemser, 1925) and a shortening of the relatively refractory period (Strughold and Jörg, 1933). This leads to increased patellar reflexes in man and animals. On the other hand, Cobb and Schmidt and their co-workers found that hyperpnea leads to a decreased blood supply of the brain through vasoconstriction, which is not compensated for by the systemic blood pressure, since it remains either unchanged or drops slightly. The effects observed in our experiments are best understood by the not unreasonable assumption that the specific effects of decreased CO_2-tension in the blood are more than overcompensated by the vasoconstriction. We come, therefore, to the conclusion that the effects of hyperpnea on hearing are in the last analysis effects of O_2-lack.

In this respect observations of Cobb and Fremont-Smith (1931) in man are of great importance. They showed that, whereas CO_2-excess increases the pressure of the cerebro-spinal fluid, hyperpnea lowers it. In contrast to the quick restoration of the normal pressure level after readmission of air following the inhalation of CO_2, it was found that the pressure remained diminished for 7 minutes and more after hyperpnea was discontinued. This seems to indicate that the vasoconstriction caused by hyperpnea is only slowly reversible and accounts for the distinct after-effects of hyperpnea on hearing. It may, therefore, well be that all the effects of hyperpnea observed in our experiments can be explained fully on the basis of the vasomotor changes. It seems, however, not wise to make too sharp a distinction between the cellular effects of O_2-lack in our experiments with inhalation of low O_2-mixtures and the vasomotor effects of hyperpnea,

because it is to be expected that a sufficiently prolonged hyperpnea will result in cellular changes due to sustained vasoconstriction.

Comparing the results of our work with those of other investigators, it may be stated that Lewis (1918 and 1919) and Bagby (1921) were unable to observe any changes in hearing due to O_2-lack except immediately before collapse. The differences are probably due to the fact that these authors applied the tests in routine experiments, whereas our observations were performed on persons trained for several weeks or months prior to the main group of experiments described in this paper. Concerning CO_2-excess or CO_2-lack, no experimental work seems to have been reported in the literature except for some statements of Rosett (1924), who finds that during hyperpnea the sensitivity to pain and deep pressure was increased. But it is quite obvious, from his description, that this increased response on the part of the experimental subject was not due to a change in the excitability but to an alteration which concerned particularly "affective reactions" of the experimental subject. When weak stimuli were used a diminution in response (although without quantitative measurements) was observed by him.

The experiments of Brody and Dusser de Barenne (1932) on the excitability of the motor cortex show that it is difficult to obtain any effect with hyperventilation. On local application of strychnine, however, hyperventilation did produce increased excitability to electrical stimuli. Whether this effect is due to subcortical or cortical effects may be uncertain. In addition one must always take into account that the effect of hyperpnea on cortical excitability (motor or sensory) is due to the interaction of at least two factors: 1, the effect of increased OH-concentration causing increased excitability; 2, the depressing effect of O_2-lack due to vasoconstriction accompanying hyperpnea. The final results, therefore, may vary according to the special conditions and the quantitative reactivity of the tissue to these factors.

CONCLUSIONS

If air with oxygen content of about 10 per cent or less is inhaled for 10 to 30 minutes a decrease in the hearing threshold is observed which, dependent on the duration of the experiment, the concentration of the oxygen and the sensitivity of the experimental subject, may last up to several hours. CO_2 in concentrations of 4 to 8 per cent in air of normal O_2 content also causes a diminution in hearing which, however, is more quickly reversible. After a period of voluntary hyperpnea lasting from 3 to 6 minutes a considerable decrease in hearing is observed during the period of apnea and afterwards, until gradually the original threshold is obtained. In all three groups of experiments a supernormal phase may

temporarily be observed when normal air is readmitted. The changes in hearing seem to be due to chemical cellular alterations in the central nervous system itself.

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ON THE RENAL EXCRETION OF UREA[1]

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Of the numerous facts concerning the renal excretion of urea the following will be retained as sufficiently well established to warrant theoretical treatment:

Fact 1. The rate of excretion per unit plasma concentration increases continuously with diuresis up to a narrow range of moderate rates of urine flow. This fact was given explicitly by Austin, Stillman and Van Slyke (1921).

Fact 2. For larger diuresis the rate of urea excretion per unit plasma concentration is independent of urine flow. This fact is due to several investigators beginning with the work of Marshall and Davis (1914). (For historical development see Möller, McIntosh and Van Slyke, 1928.)

Fact 3. The rate of excretion per unit plasma concentration depends also on protein intake. This fact we owe to Addis and Drury (1923a) and especially to Jolliffe and Smith (1931a), although the fact itself can be discovered in the early observations on the dog in the paper of Austin, Stillman and Van Slyke (1921).

The rate of excretion per unit plasma concentration has been called "clearance" by Möller, McIntosh and Van Slyke (1928). If the rate of excretion is represented by y and the plasma concentration by x, the three facts mentioned before can be symbolized by

$$(y/x) = \varphi\,(v, d) \tag{1}$$

where φ stands for some unknown function of diuresis, v, and diet, d. Since the physiological changes in urine flow are or can be made short in duration, relative to the changes induced by diet, it will be possible to determine the variation in (y/x) with diuresis, for a given stationary or average effect of diet. Under these conditions, equation 1 becomes

$$(y/x) = f(v) \tag{2}$$

In this paper I shall try to determine the form of the function f in equation 2. Data available at present on the effect of diet are insufficient to

[1] An abstract of part of this paper was presented at the Detroit Meeting of the American Physiological Society, April 11, 1935.

determine the function φ of equation 1. However, I shall make use of the interesting fact, also brought out by Jolliffe and Smith (1931a), that the lowering in the clearance produced by a cracker meal diet is accompanied by a shift in the range of diuresis at which the clearance becomes independent of urine flow.

From facts 1 and 2 above it is to be expected that as diuresis increases the clearance would become asymptotically equal to a constant, yet none of the expressions so far proposed for it satisfy this expectation. For instance, the equation of Ambard (Ambard and Weill, 1912) can be written

$$\frac{y}{x} = ax^{\frac{1}{2}}v^{\frac{1}{2}} \tag{3}$$

that of Austin, Stillman and Van Slyke (1921),

$$\frac{y}{x} = bv^{\frac{1}{2}} \tag{4}$$

and that of Walker and Rowe (1927),

$$\frac{y}{x} = cx^{.38}, \tag{5}$$

none of which have an asymptote. All the above equations can be put in the form

$$y = px^{\alpha}\, v^{\beta} \tag{6}$$

but it will be enough to consider equation 4, since the computation of the constants in equation 6 (Conway and Kane, 1929), from data in the literature, yields values sufficiently close to those of the formula of Austin, Stillman and Van Slyke (1921).

The equation for the clearance at large urine flows may be written

$$y/x = A \tag{7}$$

It will be seen that the curve represented by equation 4 will not join the curve of equation 7 along a common tangent, but will intersect it at a definite angle. Once the parameters b (equation 4) and A (equation 7) have been ascertained, by the data or otherwise, the point of intersection is definitely determined. The diuresis corresponding to it has been called "augmentation limit" by Austin, Stillman and Van Slyke (1921). These authors take, therefore, the curve of equation 4 from $v = 0$ to the point of intersection with the curve of equation 7, to represent the ratio, now called clearance, at low or moderate diuresis, and the curve of equation 7 from the augmentation limit on, to represent the clearance for large urine flows.

However well justified from an empirical point of view, this procedure is theoretically unsatisfactory. It leads, moreover, to a few inconsistencies, which I shall indicate.

The first one refers to the dimensions of the constants b and A. The dimensions of A are those of flow $[l^3t^{-1}]$, like v itself, while the dimensions of b are $[l^{\frac{1}{2}}t^{-\frac{1}{2}}]$. Now, Möller, McIntosh and Van Slyke (1928) call A the maximum clearance, and b the standard clearance, but from the dimensional standpoint it is clear that if A is a clearance, b is not. The argument that b has the dimensions of flow at $v = 1$ is not valid, because this will depend on the units in which the flow is determined, and the choice of units is evidently arbitrary.

A second inconsistency appears when the "standard clearance" is calculated in young children or in dogs, in whom the augmentation limit is less than 1 cc. per minute. For in this case, although the clearance (y/x) is smaller at diuresis below the augmentation limit than above this limit, as it should be, the standard clearance b is larger than the *maximum* clearance A. For example, in table 1 (McIntosh, Möller, and Van Slyke 1928), the maximum clearance of the first, second, third, fifth and sixth child is, respectively, 19.8, 27.9, 18.3, 24.2 and 38.9, while the standard clearance is, in the same order, 45.0, 35.9, 38.7, 26.9 and 40.8. Similarly, in Jolliffe and Smith's paper (1931b) the maximum clearance of the dog is, on an average, 58.0, and the standard clearance 92.8, both calculated per unit of body surface. The situation would not be improved by saying that the standard clearance is the clearance referred to the diuresis $v = 1$, because if the augmentation limit is less than 1, let us say, 0.4 cc. per minute, as in the dog, equation 4 would not apply at diuresis greater than 0.4 cc. per minute, and consequently the ratio (y/x) calculated from equation 4 at $v = 1$ would be meaningless. As a matter of fact, with such an interpretation, the two numbers given by Jolliffe and Smith (1931b) would mean that the average dog has two clearances at $v = 1$, a maximum clearance of 58.0 and a standard clearance of 92.8.

In order to avoid the foregoing difficulties I have proceeded in a different way.

Equation 7 may be written

$$y = \mathring{A}\, x \tag{8}$$

This means that the excretion of urea at large diuresis is of the same form as that of exogenous creatinine and xylose at any diuresis (Dominguez and Pomerene, 1934a, 1934b). Let us assume that at low urine flow urea is absorbed in the kidney itself. Then the rate of excretion y, as determined in the ordinary way, may be regarded as the resultant of two operations: first, the rate s at which urea is poured into the lumen of the

tubules, and second, the rate r at which urea is absorbed from the lumen of the tubules. In symbols

$$y = s - r \tag{9}$$

at any diuresis.

In order to determine y it is necessary to make some assumptions concerning the nature of the two rates s and r. The two assumptions are:

1. At any diuresis, the rate at which urea is removed from the blood is proportional to the concentration in the plasma, that is,

$$s = A\,x \tag{10}$$

2. The rate at which urea is absorbed varies with diuresis in such a way that the instantaneous change in the rate of absorption r with respect to diuresis v, is proportional to the rate of absorption r. In symbols,

$$\frac{dr}{dv} = -kr \tag{11}$$

where k is a constant. The minus sign indicates that the rate of absorption decreases as diuresis increases.

The integration of equation 11 gives

$$r = c\,e^{-kv} \tag{12}$$

Substituting s and r from equations 10 and 12 in equation 9, we get

$$y = A\,x - c\,e^{-kv} \tag{13}$$

and since at $v = 0$, $y = 0$, it follows that

$$c = A\,x \tag{14}$$

Therefore,

$$y = A\,x\,(1 - e^{-kv}) \tag{15}$$

or in terms of the ratio (y/x),

$$(y/x) = A\,(1 - e^{-kv}) \tag{16}$$

at any diuresis.

From equation 10, A has the dimensions of flow, and from equation 11, k has the dimensions of the reciprocal of flow. Therefore, the dimensions of equation 16 are satisfied at any diuresis. It should be noted that the product (Ak) has the dimensions of a pure number, a result which will be used later.

Equation 16 shows that as diuresis increases, the rate of excretion per

unit plasma concentration (clearance) increases asymptotically to a mean value A, provided the effect of diet is stationary. The notion of an augmentation limit drops out. In practice, however, a region of v exists in which the clearance is practically indistinguishable from its asymptotic value A. The left boundary of this region (in an ordinary Cartesian diagram) may still be called "augmentation limit," but the mean value A will be designated by "asymptotic" or "stationary clearance," in preference to maximum clearance, because the expression for the clearance (equation 16) has no maximum.

TABLE 1

Urea clearance C in man. Data by Möller, McIntosh and Van Slyke (1928)

v	C	n	NUMBER OF SUBJECTS	S	k.log e	Δ_1	b	Δ_2
0.308	22.02	4	4	1.765	0.527	−1.90	39.68	−5.27
0.518	38.94	11	8	1.704	0.673	+3.55	54.10	+3.55
0.683	47.16	17	11	1.808	0.702	+4.77	57.06	+6.52
0.867	48.33	7	5	1.718	0.578	−0.23	51.90	+2.55
1.057	48.63	9	6	1.757	0.480	−4.89	47.30	−1.92
1.288	54.58	6	3	1.735	0.500	−3.49	48.09	−1.22
1.513	62.73	3	3	1.687	0.631	+1.40	51.00	+2.25
1.675	61.30	6	4	1.660	0.526	−1.84	47.36	−2.34
1.815	68.35	2	2	1.675	0.828	+3.93	50.73	+2.11
2.047	63.67	3	3	1.627	0.493	−2.39	44.50	−6.68
2.420	65.77	3	3	1.617	0.482	−2.07		(−10.72)
≥2.60	70.57	33	6	1.728				

v = mean diuresis, cubic centimeters per minute.
C = mean clearance, cubic centimeters per minute.
n = number of observations.
S = body surface, weighted mean, square meters.
k = from equation 16 with A = 70.57.
Δ_1 = difference between observed clearance and clearance calculated from equation 17.
b = from equation 4.
Δ_2 = difference between observed clearance and points on the curve $C = 49.17\sqrt{v}$.

The assumptions leading to equation 16 have been made very broad in order to embrace several possible schemes of renal physiology, in so far as the function of the structural units of the kidney is concerned. The identification of the rates s and r of equation 9 with the scheme of glomerular filtration and tubular absorption is not possible at the present time.

Urea clearance in man. The published data of Möller, McIntosh and Van Slyke (1928) were grouped as shown in table 1 in order to test the applicability of equation 16 to the mean clearance in man. The data

are not as numerous as could be desired, but they have the advantage of giving information on the size of the subject. An interval of diuresis of 0.20 cc. per minute was taken as unit of grouping, closing the interval on the left side, thus $0.20 \leqslant v < 0.40$. From a diuresis of 2.60 up to 16.25 cc. per minute the observations yield a mean clearance equal to 70.57, which was used as the value of A. The numerical work is given in table 1. The mean value of k is 1.344. Equation 16 with the values of A and k for man becomes

$$C = 70.57\ (1 - e^{-1.344v}) \qquad (17)$$

With the help of this equation the theoretical values of the clearances were then calculated at each diuresis and the differences between the observed and calculated clearance (residuals Δ_1) computed. The variance (sum of the squares of the residuals divided by the number of points minus one) is 10.5898.

For the purpose of comparison, the constant b of equation 4 was also calculated from the first 10 means, as shown in table 1, and the residuals Δ_2 were determined as before. The variance was found to be 17.1306, showing that the equation of Austin, Stillman and Van Slyke (1921) fits the mean data of Möller, McIntosh and Van Slyke (1928) at low and moderate diuresis less well than equation 16 at any diuresis (see fig. 1). There is no correlation between the deviations from the mean body surface and the residuals corresponding to equation 17, so that the large irregularities in the mean clearances are probably all due to the small number of observations.

In view of the previous analysis of Conway and Kane (1929) on earlier observations on urea excretion, the fit of equation 16 to the data of Möller, McIntosh and Van Slyke (1928) was not anticipated, because this equation does not fit the means of Conway and Kane as well as equation 4. The problem with which I am concerned here is not one of fitting a curve to a set of points. If the stationary value of the clearance at large diuresis is considered as established, then a relation of the form of equation 6 cannot be accepted, regardless of whether it fits a group of observations in an arbitrarily restricted range of diuresis. Besides, the data collected by Conway and Kane are not entirely homogeneous, since their figures include data on fasting subjects and on others "where there was apparently much variety in the water and nitrogen intake." Under these circumstances the danger of introducing systematic deviations could hardly be avoided.

Urea clearance in the dog. For the clearance of the dog I have made use of a few published data, as will be indicated below, and especially of the numerous observations of Dr. H. Goldblatt, of the Department of Pathology, Medical School, Western Reserve University, which he has very kindly put at my disposal. Altogether Doctor Goldblatt's data consist of

108 clearances at diuresis larger than 0.5 cc. per minute and 364 clearances below 0.5 cc. per minute, not including data in which the body surface of the animal was not known. These observations were carefully examined, all the ratios recalculated, and the body surfaces computed by formula 1 of Cowgill and Drabkin (1927). The mean clearance of the first series of 15 dogs, at diuresis larger than 0.5 cc. per minute, is 38.1, with a mean body surface = 0.691 square meter. In the second series of 9 dogs, the mean clearance = 31.0, and mean body surface = 0.629.

The mean clearance and mean body surface of each dog were next determined in order to estimate the correlation between the two measurements.

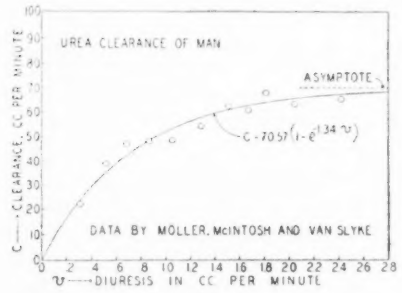
UREA CLEARANCE OF MAN
ASYMPTOTE
C = 70.57 (1 - e^-1.34 v)
DATA BY MÖLLER, McINTOSH AND VAN SLYKE
C — CLEARANCE, CC. PER MINUTE
v — DIURESIS IN CC PER MINUTE

Fig. 1

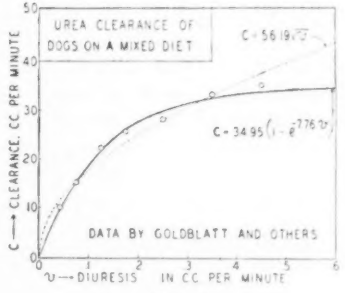
UREA CLEARANCE OF DOGS ON A MIXED DIET
C = 56.19 √v
C = 34.95 (1 - e^-7.76 v)
DATA BY GOLDBLATT AND OTHERS
C — CLEARANCE, CC. PER MINUTE
v — DIURESIS IN CC. PER MINUTE

Fig. 2

Fig. 1. Urea clearance of man. The circles represent the mean clearances of Möller, McIntosh and Van Slyke (1928) after the grouping indicated in table 1. The smooth curve has been calculated from the equation $C = 70.57\ (1 - e^{-1.34v})$. Part of the asymptote $A = 70.57$, stationary clearance at large urine flow, has been indicated by a broken line.

Fig. 2. Urea clearance of dogs maintained on a mixed diet. Data from the sources indicated in the text. Each circle represents the mean clearance of a number of observations (see table 2) in a given range of diuresis. The solid curve corresponds to equation 16 with $A = 34.95$ and $k = 7.76$. The asymptote of the curve has not been drawn in order to avoid confusion. The broken line corresponds to equation 4, with b ("standard clearance" of Möller, McIntosh and Van Slyke) equal to 56.19.

Fifteen pairs of observations were obtained from the first series and nine from the second. Five more pairs were added from the data of Jolliffe and Smith (1931b) and Jolliffe, Shannon and Smith (1932), after grouping the observations per dog.

The correlation between the clearance at $v \geqslant 0.5$ cc. per minute and the body surface of the dog is significant (correlation = 0.5608, number of pairs of observation = 29, see Fisher, 1928, table V. A). The two regression equations are

$$S = 0.506 + 0.00455\ A \tag{18}$$

$$A = -9.94 + 69.07\ S \tag{19}$$

the range of S being between 0.50 and 0.82 m^2.

For the part of the curve between $v = 0$ and $v = 0.5$ cc. per minute, I have put the (unpublished) data of Goldblatt together with those of Ralli, Brown and Pariente (1931), and of Jolliffe and Smith (1931b), after a preliminary rough grouping had shown that the four series follow the same trend. The whole material consists of 432 observations as follows:

	Number of dogs	*Mean body surface*	*Number of observations*
Goldblatt's 1. series	17	0.673	150
Goldblatt's 2. series	16	0.612	214
Jolliffe and Smith	7	0.700	19
Ralli, *et al*	9	0.563	49
	49	0.637	432

If the data are grouped by intervals of 0.1 square meter of body surface and also by intervals of 0.05 cc. per minute of urine flow, the number of observations in most of the groups is found very small. Even if the interval of diuresis is increased to 0.1, between 0.2 and 0.5 cc. per minute, the results are not much better. It is impossible, therefore, to get any idea of the correlation between body size and clearance at low diuresis, but it is possible to derive information on the mean clearance per unit of diuresis for the whole group of dogs. The mean clearances were grouped, therefore, first, by taking the mean of the means per unit of body surface, and second, by multiplying by the number of observations in each compartment (weighted mean). To each mean clearance of the first grouping there corresponds an associated mean body surface and to each mean clearance of the second grouping a weighted mean body surface.

The numerical work is presented in tables 2 and 3. The intervals of diuresis are equal to 0.05 cc. per minute from zero to 0.2, and 0.10 from then on. The value of v in the tables represents the middle of the interval, except the first one. In this interval, v is actually the mean diuresis of the group, since there are no observations below 0.025 cc. per minute. In the other intervals the means are so close to the middle of the intervals that it is immaterial which ones are used.

The value of A for the first grouping was calculated from equation 19, taking $S = 0.650$. The mean value of k is 7.76. The theoretical clearances were then calculated from the equation

$$(y/x) = 34.95\ (1 - e^{-7.7tv}) \tag{20}$$

and the residuals Δ_1 computed, as shown in table 2. The variance is 0.9889. For comparison the constant b of equation 4, and the corresponding residuals were also computed (see table 2). The variance in this case is 2.5693, indicating a less good fit than equation 16. Analogous calculations with the data of the second grouping yield the equation

$$(y/x) = 36.00\ (1 - e^{-7.28v}) \tag{21}$$

with residuals as shown in table 3. The variance is 1.0535, not much different than that from equation 20, while the variance obtained from equation 4, "standard clearance" b, is 3.7519. Equation 16 is, therefore, verified by independent observations on dogs maintained on a mixed diet, for a wide range of diuresis (see fig. 2).

TABLE 2

Urea clearance in the dog (mixed diet). First grouping

v	C	S	k log e	Δ_1	b	Δ_2
0.042	10.00	0.606	3.4848	+0.28	48.80	−1.52
0.075	15.08	0.650	3.2703	+0.01	55.06	−0.31
0.125	22.28	0.650	3.5255	+0.58	63.02	+2.41
0.175	25.66	0.650	3.2882	−0.46	61.34	+2.15
0.25	28.17	0.650	2.8488	−1.75	56.34	+0.07
0.35	33.31	0.650	3.7966	+0.67	56.30	+0.07
0.45	35.22	0.651		+1.34	52.50	

k = from equation 16 with A = 34.95.

Δ_1 = difference between observed clearance and clearance calculated from equation 19.

Δ_2 = difference between observed clearance and points on the curve $C = 56.19\sqrt{v}$.

Other symbols as in table 1.

TABLE 3

Urea clearance in the dog (mixed diet). Second grouping

v	C	S	n	k log e	Δ_1	b	Δ_2
0.042	8.44	0.528	16	2.7618	−1.05	41.18	−3.03
0.075	15.65	0.607	112	3.3029	+0.50	57.14	+0.32
0.125	22.06	0.630	95	3.2965	+0.54	62.40	+2.27
0.175	24.57	0.638	75	2.8472	−1.37	58.73	+1.15
0.25	29.10	0.679	75	2.8695	−1.07	58.20	+1.11
0.35	34.45	0.686	39	3.9015	+1.26	58.23	+1.33
0.45	35.60	0.661	20	4.3437	+0.26		

C = clearance, cubic centimeters per minute, weighted mean.

S = body surface, square meter, weighted mean.

k = from equation 16 with A = 36.00.

Δ_1 = difference between observed clearance and clearance calculated from equation 20.

Δ_2 = difference between observed clearance and points on the curve $C = 55.98\sqrt{v}$.

Other symbols as in table 1.

The published data on a meat diet and in the fasting state are insufficient to establish the applicability of equation 16 to the excretion of urea under those circumstances. There is, however, no *a priori* reason why it should not apply. On the assumption that it does, the equation should be re-

garded as representing a two-parameter family of curves, within the region of existence of the clearance. But the observations of McIntosh, Möller and Van Slyke (1928) on the clearance of the child (effect of growth) and those of Jolliffe and Smith (1931a) on the clearance of the dog (effect of diet) indicate that a change in the value of the stationary clearance A is accompanied by a shift in the augmentation limit. The two parameters A and k are, therefore, not free, but bound to each other in some way that limits their simultaneous variation in the sense observed by the above authors. It is possible to arrive at some such relationship by considering the limiting concentration ratio of urea.

The limiting value (Ak). If the concentration of urea in the urine is designated by u, in the same units as the concentration in the plasma, x, equation 16 can be written

$$\frac{u \cdot v}{x} = A(1 - e^{-kv}) \tag{22}$$

Collecting the v terms on the right-hand side of the equation we get

$$\frac{u}{x} = \frac{A(1 - e^{-kv})}{v} \tag{23}$$

The limit of the expression on the right-hand side as v approaches zero is equal to Ak, therefore,

$$\lim \frac{u}{x} = Ak \tag{24}$$

This means that the concentration of urea in the urine per unit plasma concentration approaches a limit as diuresis diminishes. It should be noted that the dimensions of equation 24 are satisfied.

The other equations proposed for the excretion of urea do not lead to any limiting value. For instance, the ratio $\left(\frac{u}{x}\right)$ from equation 4,

$$\frac{u}{x} = \frac{b}{\sqrt{v}}$$

would grow without limit as v diminishes. The limit of $\left(\frac{u}{x}\right)$ should not be confused with the "concentration limite" of Ambard and Papin (1909), later called "concentration maxime" by Ambard and Weill (1912). The latter refers to the concentration in the urine, u, but, from equation 23, no limit exists for u independently of x.

In the ideal limit, the value (Ak) appears as a state of equilibrium between the plasma and the urine, and such equilibrium may be expected to remain undisturbed by purely physiological variations in the activity of

the kidney. In particular, if the effects of diet belong in this category and we use the subscript d to indicate a given diet, we would be led to write

$$A_d \cdot k_d = m, \tag{25}$$

m being a constant independent of diet, as the simplest inverse relation between A and k. This hypothesis would immediately account for the shift in the augmentation limit with changes in A. Because, by differentiating equation 16 with respect to v, and writing C for (y/x), thus

$$\frac{dC}{dv} = (Ak)e^{-kv}, \tag{26}$$

we see that the slope of the curve will become practically zero for larger diuresis when k is small than when k is large. In other words, the range of asymptotic approach (augmentation limit) will occur at larger diuresis when A is large, as on a meat diet, than when A is small, as in fasting. And this, it will be remembered, is the fact observed by Jolliffe and Smith (1931a). A similar reasoning applies to the observed change in clearance and augmentation limit in the child. Unfortunately the data bearing on both these points are too scanty for statistical treatment, so that equation 25, however suggestive, remains hypothetical.

It should be kept in mind that if the existence of a relation between A and k is imposed by empirical facts not introduced in the development of equation 16, the form of this relation does not have to be necessarily that of equation 25. Should experiments designed for the purpose indicate a different relation, the fact would not in itself invalidate equation 16.

The value $(A\,k)$ can be considered from another point of view. Since the limit of the concentration ratio (u/x) is in some regards a measure of the power of the kidney to concentrate urea, it seems as if a small carnivorous animal would be at a disadvantage if his kidneys could not concentrate more than those of an omnivorous or herbivorous animal. The only data available on this point are those on man and on the dog. From equation 17, Ak is equal to 95 for man, and from equation 20, Ak is equal to 271 for the dog. In other words, the kidney of the dog has a concentrating power about three times as large as that of man.

The same line of thought leads to a suggestive reason for the increment in the clearance with an increment in the protein intake.

In a previous paper (Dominguez and Pomerene, 1934a), it was shown that, with a constant A, ingested creatinine disappears from the blood exponentially with the time, after the absorption by the bowel has practically ceased. From this fact it was proved (Dominguez, 1934) that the constant of elimination α is given by

$$\alpha = \frac{A}{V} \tag{27}$$

where V is the volume of distribution of creatinine in the body at the same concentration as the plasma. Since at large diuresis urea is excreted according to the same law as creatinine, it is clear that, under the same circumstances as before, equation 27 should also hold for urea. Because urea is more diffusible than creatinine, it is not likely that the volume of distribution of the former should be smaller than that of the latter. On the other hand the clearance A of urea is known to be smaller than that of creatinine. It follows from equation 27 that the constant of elimination of urea must be smaller than that of creatinine.

From preliminary experiments (unpublished data; Dominguez, Goldblatt and Pomerene), the value of α on a dog weighing 20 kgm. is, for creatinine, approximately 0.18 when the time is measured in hours. This means that every 3.85 hours the concentration in the plasma is one-half of its value at the beginning of the period. Now, the value of A for creatinine in the same dog is about 49 cc. per minute, and its value for urea, at large diuresis, is approximately 32. Therefore, on the assumption that the volumes of distribution are the same, the constant of elimination of urea should be, in this dog, equal to 0.118, or smaller, if its corresponding V is larger than that of creatinine. Consequently, the half period of the fall in urea concentration is 5.87 hours. Or, in different words, at the end of 24 hours the urea concentration in the plasma would be about 5.9 per cent of its initial value. If the ingestion of urea is repeated every 24 hours, it will be apparent that, unless the constant of elimination increases in value, the dog would slowly but surely enter into a state of urea retention. If instead of the ingestion of urea we imagine that the urea to be eliminated is derived from protein intake, we know that in a relatively short time a state of equilibrium is reached, the so-called nitrogen equilibrium. This can be accomplished only by increasing the value of the constant of elimination α. From equation 27, α can increase either by diminishing V or by increasing A, and since V (which is proportional to the body weight) does not diminish, and, in fact, may increase by the process of eating, it appears that the only way protein intake can speed up the elimination of urea is by increasing the value of the clearance A.

It should be emphasized that in the above reasoning the dog has been placed in a favorable position, because I have assumed nitrogen equilibrium, a diuresis not less than 0.4 cc. per minute, and besides I have neglected *all* the urea formed during absorption. Moreover, a value of $A = 32$ cc. per minute corresponds to the mixed diet level. Had I assumed a fasting level of 23 cc. per minute for A, the constant of elimination would have been necessarily smaller and the resulting plasma concentration at the end of 24 hours larger than 6 per cent of its initial value.

From the foregoing considerations the increment in the clearance with protein digestion appears as a nice self-adjusting device, whereby the

time of the elimination of urea is shortened and an undesirable retention of waste products prevented. In fact the very existence of a nitrogen equilibrium rests on this phenomenon.

That urea is not the cause or its removal the purpose of the increase in the clearance is amply demonstrated by the failure of urea to raise its own clearance even at very large plasma concentrations (Drury, 1923). For this reason administered urea can be used in the study of urea excretion. But all researches on nitrogen metabolism in which a constant rate of excretion per unit plasma concentration has been assumed, will require revision.

The efficiency of urea excretion. From the standpoint of urea excretion the efficiency of the kidney may be defined, in analogy to mechanical efficiency, as the ratio of the urea supplied the kidney in a given time to the urea put out by the kidney in the same time. The quantity supplied is equal to the flow in the renal arteries multiplied by the concentration of urea in the arterial blood. The quantity removed from the body by the kidneys is equal to the flow of urine multiplied by the concentration of urea in the urine. If we designate the efficiency E and the arterial blood flow by B, we have by definition

$$E = \frac{v \cdot u}{B \cdot x} \tag{28}$$

In this equation equi-dimensional quantities are supposed to be measured in the same units, and since both numerator and denominator have the same dimensions the efficiency is a pure number (see however Möller, McIntosh and Van Slyke, 1928, where the clearance itself is called efficiency).

Substituting in equation 28 the value of $v.u = y$ from equation 15, we get

$$E = \frac{A(1 - e^{-kv})}{B} \tag{29}$$

For large diuresis the efficiency becomes simply

$$E = \frac{A}{B} \tag{30}$$

Since A is here a mean value, it would be enough to postulate a mean blood flow through the kidneys in order to obtain a mean efficiency. In the case of the dog, I have taken the mean of the mean figures for blood flow through one kidney which have been conveniently tabulated by Van Slyke, *et al.* (1934) from many different sources. The mean is 120.7, or, if two very small values are omitted, 131.2 cc. per minute. Introducing in equation 29 the values of A and k from equation 20 we get an efficiency

varying from 0 to 14.5 per cent (or 13.3 per cent if the larger mean blood flow is taken), as diuresis increases from 0 up to any value beyond the augmentation limit. This figure means little by itself because the value of A depends on the diet, and little is known of the effect of diet on the blood flow through the kidney. Whatever it might be, a blood flow of 2 cc. per minute per gram of kidney will represent a flow of 600 cc. per minute through the two kidneys of an average adult man, and this flow together with a clearance of 70.6 cc. per minute gives an efficiency $E = 11.8$ per cent for diuresis above the augmentation limit. Or, if the figures of Burton-Opitz (quoted by Wiggers, 1923) are applied to man, namely, 150 cc. per 100 grams of kidney, we get an efficiency of 15.7 per cent.

The urea clearance of the rabbit, from the observations of Addis and Drury (1923b) on 18 rabbits, is about 4.65 cc. per minute, while it is 6.27 in the experiments of Drury (1923) on five rabbits weighing from 2.2 to 2.5 kilos. On the other hand, the blood flow through the kidneys is on an average 2 cc. per minute per gram of kidney (Kay and Sheehan, 1933), and since, according to the same authors, the mean weight of the two kidneys is 17 grams in a group of animals with a mean body weight of 2.53 kilos, the efficiency of urea excretion in the rabbit is, on an average, equal to 13.7 per cent. The figures of Drury (1923) would yield an efficiency of 18.4 per cent if the mean blood flow of 34 cc. per minute were assumed, but it is enough to take a blood flow of 2.7 cc. per minute to bring the efficiency in this case to the same level of 13.7 per cent. From table 12 of Kay and Sheehan's paper (1933), it appears that a blood flow between 1 and 3 cc. per minute per gram of kidney was recorded 127 times in a total of 200 observations, so that a flow of 2.7 cc. per minute is not excessive.

The efficiency is, therefore, of the same order of magnitude in the dog, the rabbit and man.

A quantity similar to the efficiency E (in practice if not in principle) has been recently used by Van Slyke *et al.* (1934) to estimate the blood flow through the kidneys. The equation used by these authors for the blood flow B' is, in the symbols of this paper,

$$B' = \frac{y}{x_a - x_v} \tag{31}$$

where the subscripts a and v refer to concentration of urea x in the artery and vein respectively. Dividing numerator and denominator on the right-hand side of equation 31 by x_a, we obtain

$$B' = \frac{A}{E'} \tag{32}$$

where $E' = (x_a - x_v)/x_a$ is the extraction ratio of Van Slyke. (The name originates with Dunn, Kay and Sheehan, 1931.) The formal analogy of equation 32 to equation 30 is evident.

By substituting the mean value of A and the mean value of E' from the data of Van Slyke a value is obtained for B' which agrees well with direct measurements of flow through the renal artery. But if this result applies to the mean values, it does not follow that the same holds for any estimated value of blood flow. Since the blood flow was not measured, it is clear from equation 32 that the values of B' will vary with variations in A and E', and it would be meaningless to say that variations in A are produced by variations in B'. Therefore, the proposition that spontaneous variations in the urea clearance parallel variations in renal blood flow, cannot be proved from the data of Van Slyke, *et al.*

SUMMARY AND CONCLUSIONS

By assuming that at low diuresis the diminution in the urea clearance is due to absorption of urea in the kidney itself, and by giving to this assumption a mathematical expression, it is possible to throw the relation between clearance, C, and diuresis, v, in the form

$$C = A\ (1 - e^{-kv})$$

where A is the mean asymptotic value of the clearance as v increases, k a positive constant, and e the base of natural logarithms.

It is shown that, both in man and in the dog, *under ordinary diets*, the means of a large series of observations at low urine flow are more faithfully represented by the above equation than by the equation proposed by Austin, Stillman and Van Slyke. Since in the above expression the clearance becomes asymptotically equal to a constant, the equation represents the change in the mean clearance with diuresis, at any urine flow.

A consequence of the equation is the existence of a limiting concentration ratio for urea, the limit being equal to $A\ k$ as diuresis approaches zero. Since the limiting concentration ratio is a measure of the concentrating power of the kidney, it is pointed out that this limit is considerably larger in a carnivorous animal like the dog, than in an omnivorous animal like man.

Inasmuch as a change in the mean value A by diet (in the dog) or by growth (in the child) is accompanied by a shift in the range of asymptotic approach to A, it is demonstrated that a shift of this kind can be produced by assuming that the limiting concentration ratio is a constant independent of diet (or of growth). The quantitative verification of this assumption, however, has not yet been given.

A deep physiological significance is attached to the increase in the clearance A with protein intake. From previous considerations on the

simple case of creatinine excretion after absorption has practically ended, it is shown that a retention of urea would inevitably follow the ingestion of protein, unless protein intake raises in some way the clearance of urea. The existence of this phenomenon is essential for the establishment of a nitrogen equilibrium.

By defining the efficiency of urea excretion by the kidney as the ratio of the urea supplied the kidney in a given time to the urea put out by the kidney in the same time, it is possible to state that the efficiency equals the ratio of the mean clearance to the mean renal blood flow. The efficiency of urea excretion, computed from a mean clearance and a mean renal blood flow from independent sources, shows that the efficiency is of the same order of magnitude in man, dog and rabbit.

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THE EQUILIBRIUM TIME OF THE GASEOUS NITROGEN IN THE DOG'S BODY FOLLOWING CHANGES OF NITROGEN TENSION IN THE LUNGS[1]

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Direct measurements have not been made in man or in animals either of the gaseous nitrogen content of the whole body or of the time required to establish equilibrium between the partial pressure of nitrogen in the lungs and in the tissues when changes in barometric pressure occur.

It is generally held that the gaseous nitrogen is dissolved in the fat and the water of the body. Vernon (1907) and Campbell and Hill (1933) have shown that nitrogen is five times as soluble in fat as in water. If the body is 15 per cent of fat by weight, then the nitrogen is about equally divided between the fat and the water.

The rate of saturation of the body with excess nitrogen when the partial pressure of nitrogen in the lungs is raised, or the rate of desaturation when the partial pressure is lowered, depends essentially on the blood flow through the lungs and the tissues and hence may be considered a function of cardiac minute volume. Zuntz (1897) assumed that when the partial pressure of the nitrogen in the body is in excess of that in the lungs, a constant percentage of the remaining excess nitrogen passes into the blood per unit of time and is eliminated through the lungs. This assumption has been represented graphically by Boycott, Damant and Haldane (1908) for any tissue or part of the body through which the blood flow is uniform. Of particular importance is the determination of the rate at which the most slowly saturating tissues come into equilibrium with changes in the nitrogen tension in the lungs. From data obtained by experiments on goats subjected to high air pressures, and from men working under excess pressure, Boycott, Damant and Haldane (1908) concluded that about 5.5 hours were necessary for 98 per cent saturation or desaturation of those tissues having the slowest rate of saturation with nitrogen.

In man, measurements of nitrogen elimination from the body when pure oxygen is inhaled were made by Bornstein (1913) for periods of approxi-

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mately 7 minutes. Campbell and Hill (1931) using a modification of Bornstein's method found that approximately 200 to 300 cc. of nitrogen, or approximately one-third of the total content of the body, were eliminated during the first 9 minutes. Campbell and Hill (1933) measured the nitrogen absorbed by the brain, liver and bone marrow of goats and concluded that only 50 per cent saturation was attained in these tissues after 3 to 5 hours' exposure to excess pressures.

Valuable as these studies are for individual tissues they nevertheless do not show directly the manner in which nitrogen is absorbed or eliminated by the intact animal. In the experiments presented in this paper measurements were made on dogs, showing the rate at which nitrogen equilibrium is attained in the living, intact body when exposed to varying air pressures for varying periods of time.

METHOD. The gaseous nitrogen in the body was removed by the inhalation of pure oxygen. Since the partial pressure of the nitrogen dissolved in the body is the same as that in the alveolar air, the substitution of oxygen in the lungs for the nitrogen normally present will, if time be permitted, result in the complete elimination of all the nitrogen held by the tissues. Dogs which had been deprived of food for 40 hours were anesthetized with Dial,[3] 0.6 cc. per kgm. of body weight, injected intraperitoneally. Repeated experiments were frequently performed on the same dog.

The anesthetized dog was placed in a metal box having a lid which fitted into a mercury seal. The gas in the system was kept in constant circulation by means of a gas-tight blower, which was immersed in a bath of heavy oil to ensure further against leakage of air into or gas out of the blower. Upon leaving the box the gas passed through a can containing soda-lime for the absorption of carbon dioxide. The spirometer served as a reservoir from which oxygen was supplied to meet the metabolic requirements. Before returning to the box, the circulating gas passed through a coil immersed in ice water in order to condense out the excess moisture and to prevent an excessive rise in temperature.

After the dog had been inclosed in the box, the entire apparatus was rinsed out with oxygen. This process required 7 minutes. During this time the nitrogen which was eliminated from the body was lost. From estimations, based upon the subsequent nitrogen elimination, the fraction lost in this manner had a constant value of 30 ± 5 per cent of the total nitrogen content of the body. A check on this value was obtained from a post-mortem analysis of the fat and water content of dog D. The gaseous nitrogen content of the body was then equal to the weight of the fat and water multiplied by the solubility coefficient of nitrogen. From these data it was estimated that the nitrogen lost was 32.3 per cent of the total.

[3] The authors wish to thank Dr. Charles C. Haskell of the Ciba Company, New York City, for his kindness in furnishing the dial-urethane solution used as the anesthetic in these experiments.

The volume of nitrogen in the apparatus at any given time was determined by multiplying the percentage of nitrogen by the total volume of gas in the apparatus, allowance being made for the gas displaced by the dog's body. The volume was corrected for water vapor tension and then reduced to standard conditions of temperature and pressure. Changes in volume could be read from the spirometer scale with an accuracy of ± 10 cc. The nitrogen concentration was determined by the manometric method of Van Slyke and Sendroy (1932). By means of check analyses the analytical error was not allowed to exceed ± 0.003 per cent. In a system containing 100 liters the analytical error was therefore ± 3 cc. of nitrogen.

Four control experiments made without a dog in the apparatus for periods of 5 hours gave very satisfactory results. In every respect the regular experimental procedure was followed. Oxygen was admitted during the experiment and the necessary corrections made for the nitrogen added with the oxygen, and for the nitrogen entering through the water seal of the spirometer. In only 1 analysis out of 11 did the observed values deviate by more than 3 cc. from the calculated values.

The concentration of nitrogen in the apparatus at the termination of an experiment was less than 1 per cent. The partial pressure of nitrogen in the body under normal atmospheric conditions is about 573 mm. Under the conditions of our experiments the partial pressure of the nitrogen in the body is reduced to 7.6 mm. or less, instead of 0 mm., so that the volume of nitrogen eliminated is about 1.3 per cent less than the true value for complete desaturation. No correction has been applied since the error involved is insignificant.

EXPERIMENTAL RESULTS. Table 1 shows the nitrogen eliminated from 7 different dogs which were in nitrogen equilibrium with air at atmospheric pressure. On 3 dogs the experiment was repeated on different days. The volume of nitrogen collected when reduced to cubic centimeters per kilogram of body weight is very constant with the exception of dog A which gave up 8.4 cc. of nitrogen per kilogram of body weight, and dog G which gave up 14.0 cc. per kilogram. The former was exceptionally thin and the latter was an old dog and moderately fat. These extreme values, therefore, may be correlated with differences in the fatty content of the tissues, since nitrogen is five times as soluble in fat as in water. Although the volume of nitrogen collected from these dogs is less than the total nitrogen contained in the body, owing to the nitrogen lost during the first 7 minutes of rinsing with oxygen, we may assume that the nitrogen collected bears approximately the same ratio to the total nitrogen in the body in each case.

Figure 1 shows typical desaturation curves of 4 dogs. Similar curves were constructed for each experiment, showing the per cent desaturation correlated with time. These data are given in the last two columns of table 1.

Whether or not the desaturation time remains constant for all pressures must depend upon the capacity of the blood to carry its increased load of nitrogen. Experimental data relative to this question are afforded by curves representing the nitrogen elimination of dog D following equilibrium established at 1, 3 and 4 atmospheres of pressure. It was then compressed for 4 hours, a period of time which was 1 hour in excess of the saturation time for this dog (see D experiments, fig. 1 and table 1), assuming that saturation time equals desaturation time, as will later be shown to be the case. At the termination of the compression period the dog was decompressed in 10 seconds and immediately placed in oxygen and desaturated of its nitrogen by the method described. If the volume of nitrogen

TABLE 1

*Desaturation from 1 atmosphere of air. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected**

DATE	EXPERIMENT	WEIGHT	TYPE	TOTAL NITROGEN	NITROGEN PER KILOGRAM OF BODY WEIGHT	DESATURATION TIME	
						95 per cent	100 per cent
		kgm.		*cc.*	*cc.*	*min.*	*min.*
Nov. 13	A	8.8	Very thin	74	8.4	130	180
July 13	B-1	12.3	Thin	117	9.5	130	180
July 19	B-2	11.3	Thin	104	9.2	130	180
Oct. 17	C	11.6	Very thin	108	9.3	130	220
Oct. 4	D-1	11.9	Thin	118	10.0	134	180
Oct. 5	D-2	11.9	Thin	110	9.2	132	180
Nov. 16	D-3	12.3	Thin	124	10.0	112	154
Nov. 27	E	13.6	Thin	136	10.0	130	180
Nov. 8	F-1	13.2	Moderately fat	151	11.4	168	240
Nov. 9	F-2	13.2	Moderately fat	140	10.6	184	240
Oct. 29	G	13.8	Old and fat	193	14.0	174	240

* Since 30 ±5 per cent of the nitrogen of the body was eliminated during the first 7 minutes of rinsing with oxygen, the values for total nitrogen and nitrogen per kilogram of body weight are only 70 ±5 per cent of the total nitrogen present.

absorbed is proportional to the partial pressure of nitrogen in the lungs, then desaturation from 3 atmospheres of pressure should yield three times as much nitrogen as desaturation from 1 atmosphere, etc.; and if the rate of desaturation from 3 atmospheres of pressure is the same as the rate of desaturation from 1 atmosphere, then at all times the volume of nitrogen eliminated must be in the ratio of 3 to 1, etc.

Curve *A*, figure 2, represents the nitrogen desaturation from 1 atmosphere of pressure; curve *B*, desaturation from 3 atmospheres; and curve *C*, desaturation from 4 atmospheres. The curves are drawn through the experimental points which are indicated by 0, while the points indicated by x represent the nitrogen which would be eliminated if the volume

absorbed were exactly proportional to the increased pressure of the air breathed. Desaturation from 3 atmospheres of pressure yielded 334 cc. of nitrogen versus the theoretical value of 354 cc., giving a ratio of 2.83:1 instead of 3:1. Desaturation from 4 atmospheres yielded 500 cc. versus the theoretical value of 472, giving a ratio of 4.23:1 instead of 4:1. The discrepancies fall within the experimental error for this procedure. In another experiment of the same nature on dog C, it was found that desaturation from 4 atmospheres of pressure yielded 440 cc. of nitrogen and desaturation from 1 atmosphere of pressure yielded 108 cc. of nitrogen, the ratio being 4.14:1. These experiments indicate that the volume of

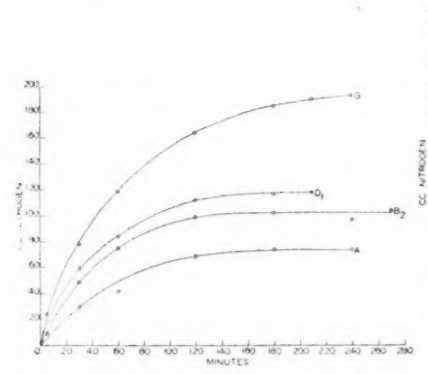

Fig. 1

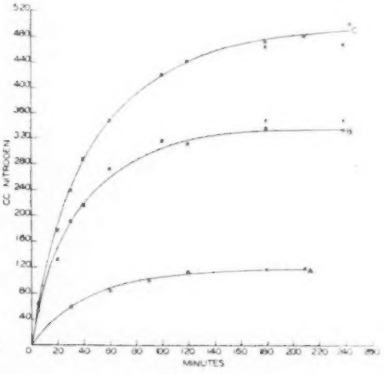

Fig. 2

Fig. 1. Desaturation from air at 1 atmosphere of pressure. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected.

Fig. 2. The nitrogen elimination of dog D saturated with air at 1, 3, and 4 atmospheres of pressure. The nitrogen eliminated during the first 8 minutes of oxygen inhalation was not collected. Curve A, desaturation from 1 atmosphere; curve B, desaturation from 3 atmospheres; curve C, desaturation from 4 atmospheres. ⊙, experimental values; X, theoretical values.

nitrogen absorbed is proportional to the partial pressure of the nitrogen in the lungs, and that the rate of desaturation from pressures of 1, 3 and 4 atmospheres is the same.

The nitrogen elimination curves following exposures to high pressures may throw some light upon the manner in which the excess nitrogen is held in the body. Is it held in physical solution or as minute bubbles? A gas which is held in physical solution in a fluid will tend to effervesce and form bubbles when the partial pressure of that gas exceeds the barometric pressure upon the fluid. At a pressure of 1 atmosphere, therefore, we should expect to find nitrogen leaving the blood as bubbles when its partial pressure exceeds 760 mm. When the bubble first forms the

partial pressure of the nitrogen in it, under normal atmospheric conditions, is 760 mm.; but as the gas in the bubble comes into equilibrium with the tissue gases—water, carbon dioxide and oxygen—the nitrogen becomes diluted until its partial pressure is approximately 627 mm. This pressure will remain constant so long as any excess nitrogen remains in the body. Under these conditions the nitrogen would pass from the tissues into the blood under a constant pressure head, and consequently at a constant rate, until the bubbles were completely absorbed. The resultant desaturation curve would tend to be a straight line whereas the true curve tends to flatten progressively, indicating a constantly diminishing yield of nitrogen per minute.

The curves presented, therefore, show that the body fluids are capable of carrying nitrogen in a state of supersaturation. Rapid decompression from 4 atmospheres of pressure results in a degree of nitrogen supersaturation of the tissues which may be measured by the difference between the initial nitrogen pressure of 2370 mm. existing in the body at the moment of decompression, and the barometric pressure of 760 mm. which immediately follows. The difference (2370 − 760 = 1610 mm.) is far too great to be explained by our present knowledge of the behavior of gases in supersaturation in vitro. We must, therefore, attribute this phenomenon to some characteristic of the body fluids which functions only in vivo.

To determine whether nitrogen passes into the tissues at the same rate that it is given up, when the pressure head is the same in both cases, the dog was completely desaturated and then exposed to air at different pressures for periods of time less than that required for complete saturation. If the total volume of nitrogen taken up during the saturation period is the same as that given off during the same period of time, then the saturation time equals the desaturation time. In figure 3, curve *A* represents the desaturation rate of dog D following a state of equilibrium with air at 1 atmosphere of pressure. The same dog, after complete desaturation, was then made to breathe air at 1 atmosphere for 67 minutes and again the nitrogen was collected by exposure to oxygen, as shown by curve *B*. If the unknown quantities of oxygen lost during the first 7 minutes in each experiment were approximately equal, so that the nitrogen collected in the succeeding 60 minutes was an indication of the relative rates of saturation and desaturation, then the ratio of saturation time to desaturation time is 92:86, or a difference in rate of only 7 per cent. This relationship is shown by the broken line. The same experiment was done under a pressure of 4 atmospheres, curve *C* representing the nitrogen collected after complete saturation and curve *D* the nitrogen collected after an exposure of 67 minutes. In this case the ratio is 348:368, or a difference in rate of about 6 per cent. This discrepancy is no greater than

we should expect to arise from the experimental errors, so that we may conclude that the saturation time and desaturation time are essentially equal.

Experiments were done to determine the desaturation time of the body following incomplete saturation with nitrogen. Dogs were first completely desaturated of the nitrogen in the body and then exposed to air for periods of time which were less than the known saturation time. The nitrogen from the partially saturated body was then collected by the procedure herein described and the desaturation curve constructed. One might

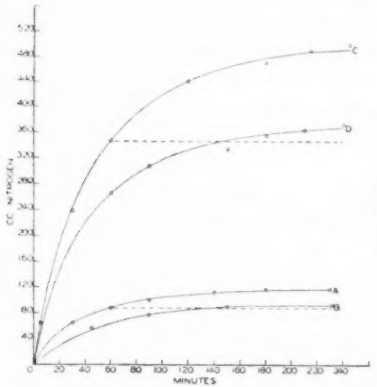

Fig. 3

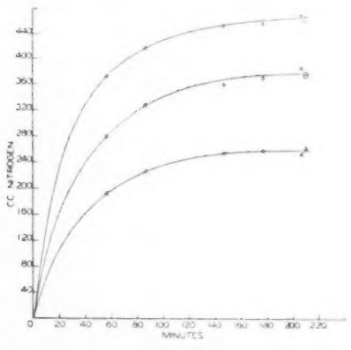

Fig. 4

Fig. 3. Saturation time compared with desaturation time. Dog D. The nitrogen eliminated during the first 7 minutes of oxygen inhalation was not collected. Curve A follows complete saturation at 1 atmosphere; curve B follows 67 minutes' saturation at 1 atmosphere; curve C follows complete saturation at 4 atmospheres; curve D follows 67 minutes' saturation at 4 atmospheres.

Fig. 4. The nitrogen elimination after incomplete saturation with air at 3, 4, and 5 atmospheres. Dog E. The nitrogen eliminated during the first 8 minutes of oxygen inhalation was not collected. Curve A follows 65 minutes' saturation at 3 atmospheres; curve B follows 65 minutes' saturation at 4 atmospheres; curve C follows 65 minutes' saturation at 5 atmospheres.

expect under these conditions that the nitrogen from the rapidly saturating tissues would be released very rapidly, and that the curve would then flatten out with extreme abruptness due to the fact that the slowly saturating tissues have been only partially saturated. According to this hypothesis, the shorter the period of saturation, the greater should be the departure from the normal desaturation curve as determined under equilibrium conditions. Our experiments show, however, that the desaturation time is constant for all degrees of previous saturation. Inspection of figure 4 will make this apparent. Curves *A*, *B* and *C* represent the nitrogen

desaturation time of dog E after exposure to 3, 4 and 5 atmospheres of pressure for 65 minutes. The time of desaturation was not 65 minutes but 200 minutes, the latter being the time which was required for this particular dog to give up its nitrogen after complete saturation. It is interesting to note that the shape of these curves resembles, in all respects, the desaturation curves of dogs which have been completely saturated.

That the desaturation time is constant, irrespective of the degree of saturation, may be more accurately demonstrated by curves which correlate per cent desaturation with time. The curve in figure 5 shows the rate at which the nitrogen is eliminated from dog D, when desaturated from a state of equilibrium with air at 1 atmosphere of pressure, in terms of percentage desaturation against time. When the same dog was exposed to

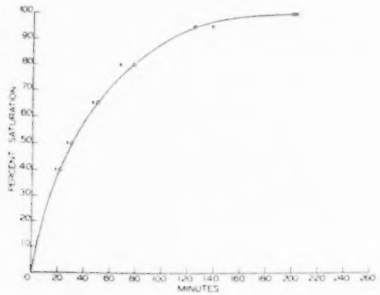

Fig. 5. The nitrogen elimination after incomplete saturation with air at 4 atmospheres. Dog D. The nitrogen elimination during the first 8 minutes of oxygen inhalation was not collected. ⊙, desaturation rate following complete saturation. X, desaturation rate following partial saturation.

4 atmospheres of pressure for 37 minutes and the desaturation rate then reduced to the same terms, it is found that the points (x) for this curve coincide with the curve for complete saturation.

The fact that the rate of nitrogen elimination from the partially saturated body coincides with that of the completely saturated body indicates that the nitrogen in the quickly saturating tissues is constantly moving into the more slowly saturating tissues, thus tending to equalize the nitrogen tension throughout the entire body at all times. For example, at normal atmospheric pressure, about 120 cc. of nitrogen were given off by dog D when completely desaturated under the conditions of our experiments. When exposed to 4 atmospheres of pressure for 37 minutes the same dog under the same experimental conditions gave off 266 cc. of nitrogen. This curve is exactly similar to that which we should expect to find if the dog had been completely saturated at an atmospheric pressure which was proportional to the nitrogen eliminated, or 2.22 atmospheres

(266/120 = 2.22). Had there been a tendency for the nitrogen tension in the rapidly saturating tissues to remain in equilibrium with the tension of nitrogen inspired, i.e., air at 4 atmospheres, while the more slowly saturating tissues were still unsaturated, then the first part of the curve should have been disproportionately steep. These findings lead to the conclusion that complete desaturation from a state of partial saturation follows the same curve, and consequently requires the same length of time as desaturation from a state of complete saturation.

SUMMARY

1. A method for measuring the nitrogen content of the body and its rate of elimination is described. The experiments were done upon dogs.

2. Under conditions of equilibrium at pressures up to 4 atmospheres absolute, the nitrogen content of the body is proportional to the partial pressure of nitrogen in the lungs, and the rate of nitrogen elimination is the same at all pressures.

3. The rate of nitrogen elimination from the completely saturated body, i.e., under conditions of equilibrium, is the same as the rate of elimination from the partially saturated body.

4. The saturation time is the same as the desaturation time.

5. Evidence is given showing that nitrogen is held by the blood and tissue fluids in a state of supersaturation to a high degree.

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THE PSYCHOLOGIC EFFECTS FROM BREATHING AIR AT 4 ATMOSPHERES PRESSURE[1]

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Air at high barometric pressures produces a narcotic effect on man. The changes which first appear at 3 atmospheres pressure are expressed in alterations of behavior, slowed mental activity, and impaired neuromuscular coördination. These effects, although previously observed, have not been attributed to the direct action of air or of the component gases, oxygen, nitrogen[3] and carbon dioxide.

Hill and Phillips (1932) reported that some of the deep sea divers (at depths of 270 to 300 feet, or 9 to 10 atmospheres absolute) had difficulty in assimilating facts and in making quick decisions, summarized as "a slowing of the process of cerebration." Severe emotional disturbances were sometimes observed and loss of consciousness reported. The men affected in this manner were regarded as psychologically unstable. Damant (1930) with reference to the paper of Hill and Phillips mentioned the "subtle change in character and behavior which comes over some men at less high air pressures," and considered that either the increased oxygen tension or impurities in the air might be responsible. Hill, according to Damant, excluded oxygen and carbon dioxide as etiologic factors.

Because of the many abnormal factors in the environment of the deep sea diver, the conclusion could not be drawn that changes in behavior and mental activity were caused by increased air pressure alone. It was of interest, therefore, to make observations on persons who were subjected to high air pressures in surroundings that were otherwise normal.

This paper presents a description of the psychic changes in air at 4 atmospheres, and the inference is drawn that the increased partial pressure of nitrogen is mainly responsible for the abnormal responses.

The observations were made in a large, well lighted pressure chamber described by Thomson, Yaglou and Van Woert (1932), on nine persons engaged in physiologic research under ordinary laboratory conditions except that the barometric pressure was raised from 1 to 4 atmospheres.

[1] This research was aided by the Miriam Smith Rand Fund.

[2] Member of the United States Naval Medical Corps.

[3] Includes argon and the rare gases.

The time of exposure to the increased pressure varied from 1.5 to 5 hours including decompression time. The temperature of the air was 25°C. and the relative humidity 50 per cent. Variations in temperature and humidity did not occur except for a period of 10 minutes during compression from 1 to 4 atmospheres and for a period of 2 minutes during decompression from 4 to 2.3 atmospheres.

The evaluation of the psychic changes was made on the basis of subjective and objective reactions of trained men engaged in animal experimentation. The usual procedures followed were cannulation of blood vessels, withdrawal of blood and cisternal fluid, collection of alveolar gas samples, and recording the blood pressure and oxygen consumption. Quantitative values have not been assigned to the changes, and their validity rests on the basis of a constant altered response of trained men when the air pressure was raised from 1 to 4 atmospheres.

OBSERVATIONS. The abnormal reactions were first felt at a pressure of 3 atmospheres. At 4 atmospheres the members of the entire group were affected in a similar manner with differences only in the degree of intensity of the responses.

Emotional reactions. As a pressure of 4 atmospheres was approached the individual was definitely aware of a feeling of stimulation, alertness and well-being—a definite euphoria. The mood was usually well controlled but occasionally expressed itself in laughter and loquacity. With a greater effort of self-control, normal conduct could be maintained.

Impairment of the higher mental processes. A slowing up of mental activity was a characteristic response. Visual, auditory, olfactory and tactile reception were not affected, but the response to these stimuli was delayed. A limitation of the power of association and a tendency toward fixation of ideas had to be counteracted. Recollection, consequently, required greater effort, and concentration was comparatively difficult. Frequent errors were made in arithmetical calculations and in the recording of data. One person had difficulty in telling time, confusing 43 minutes, for example, with 48 minutes. In the recording of time a reading of 12:15 might be written as 15:15. The responses were those of mild stupor in which greater effort and more time were necessary for accuracy.

Impairment in neuromuscular control. Increased difficulty attended the measurement of liquids and the manipulation of burettes and pipettes. The turning of stopcocks in the wrong direction was frequent. The impaired control of finer movements increased the breakage of glassware. The defect in coördination was essentially a past pointing or exaggeration of movement. The impairment, however, became negligible if slower movements were made.

In a single test at a pressure of 10 atmospheres (in another chamber) one member of the group felt a greatly increased numbness which amounted

to partial stupefaction. A simple task, the palpation of the pulse of another worker, was accomplished only with extreme difficulty. Efficient neuromuscular response was abolished.

That the changes increase in severity as the pressure is increased is shown by the responses at 3, 4, and 10 atmospheres. The retardment of mental activity is first felt at 3 atmospheres, becomes a slight handicap at 4 atmospheres, and at 10 atmospheres may render an individual helpless.

The altered responses occurred at the beginning of an exposure to increased pressure, and they did not change in intensity over periods of 3 hours. Irritability, fatigue and drowsiness were not present, but following decompression fatigue with a tendency to sleep was the usual reaction. Since this fatigue can be abolished by breathing oxygen during decompression, it is probably related to the presence of excess nitrogen possibly in the form of small bubbles.

Discussion. *Nature of the changes.* The altered responses in air at high barometric pressures can be produced also by a variety of agents which depress the higher centers. Among these are alcohol, the inhalation anesthetics, i.e., ether, nitrous oxide, narcotic drugs, and conditions which lead to anoxemia. Thus the mental responses from decreased oxygen tension at normal barometric pressure (McFarland, 1932), and at moderately high altitude (Barcroft, 1925) are similar to the responses at increased air pressures.

Since the severity of the reactions is related to the degree of pressure and ranges from slight impairment at 4 atmospheres to partial stupefaction at 10 atmospheres, it is inferred that a limiting pressure incompatible with human activity would be reached between 10 and 15 atmospheres pressure.

The consideration of the etiology of the responses associated with high air pressures brings into the discussion the effects of the increased tensions of oxygen and nitrogen.

The action of the increased partial pressure of oxygen. At 4 atmospheres the partial pressure of oxygen in the air (4 × 21 per cent) is 84 per cent of 1 atmosphere, and at 10 atmospheres of air is equivalent to 2.1 atmospheres of pure oxygen. The breathing of pure oxygen, however, at pressures of 1 to 4 atmospheres does not induce the psychic responses of air with corresponding or considerably lower oxygen tensions (Behnke, Johnson, Poppen and Motley, 1935). Oxygen breathed for a period of several hours may disturb the coördination of finer movements, but euphoria is not present. In contrast with the effect of oxygen, the symptoms at high air pressures are immediate in their onset. The increased partial pressure of oxygen, therefore, cannot be a significant factor in the etiology of the changes.

The action of the increased partial pressure of nitrogen.[4] If oxygen is

[4] Includes argon and the rare gases.

excluded, then the atmospheric nitrogen can be considered as mainly responsible for the narcotic action of air at high pressures. Although nitrogen is chemically inert, the physical property which renders this gas analogous to narcotic substances is its high coefficient of solubility in lipoid matter. Ether and nitrous oxide which induce narcosis are also chemically inert substances. The Meyer-Overton law indicates that a definite relationship exists between the power of a narcotic (derivatives of the hydrocarbon series) and its "partition coefficient," or the ratio of the solubility in fat to the solubility in water. Although the Meyer-Overton law was formulated with regard to the aliphatic narcotics, it is not unreasonable to assume that the high concentration of molecular nitrogen in the lipoids of the central nervous system decreases cell membrane permeability to produce the changes characteristic of air at high pressures.

The "partition coefficient" of ether is 4.3, that of nitrous oxide 1.89, and that of nitrogen 5.0. Nitrous oxide induces anesthesia when the concentration in the blood is 0.017 mol per liter, and ether acts in the same manner when its concentration is 0.003 mol per liter. At an air pressure of 10 atmospheres the molal concentration of nitrogen in the blood is 0.0043, and at a pressure of 40 atmospheres 0.017 mol or the equivalent of the anesthetic concentration of nitrous oxide. While the limiting pressure at which air is respirable has not been determined, it is believed that loss of consciousness might occur between 10 and 15 atmospheres pressure.

PRACTICAL CONSIDERATIONS. An artificial gas mixture for divers is essential if operations at great depths (above 300 feet) are carried out, and for caisson workers if the exposure at and higher than 4 atmospheres be continued over long periods of time. Such a mixture, of course, should limit the oxygen concentration to that in the air at sea level, and in addition should provide a rapidly diffusible, sparingly soluble gas with a low "partition coefficient." The last qualification is of the greatest importance since large quantities of gas dissolved in fat relative to the amount dissolved in water increase the danger of bubble formation on decompression. Furthermore, if our hypothesis be correct, the psychic effects produced by a gas like nitrogen with its high solubility coefficient in fat, will be minimized.

SUMMARY AND CONCLUSIONS

Air at and higher than 3 atmospheres pressure exerts a narcotic effect on man with the characteristics of euphoria, retardment of the higher mental processes, and impaired neuromuscular coördination.

At 4 atmospheres these changes can be counteracted by increased effort, but at 10 atmospheres they amount to stupefaction with greatly impaired muscular activity.

The increased partial pressure of oxygen does not account for these changes. It is inferred that the atmospheric nitrogen is the etiologic

factor, and that it acts on the nervous system because of the high solubility coefficient of this gas in lipoid substances compared with that in water.

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OBSERVATIONS ON THE VARIATION IN WATER CONTENT OF THE FECAL MATERIAL ALONG THE COLON

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It is generally accepted that the concentration of fecal material due to absorption of water takes place in the cecum and proximal colon. Roith (1) states that "The contents of the transverse colon in man are generally as firm as those in the rectum." Larson (2) in his review of the physiology of the colon, points out that "if fluids went beyond the hepatic flexure, they would interfere with the storage function of the colon." Hurst (3) asserts that while most of the water absorption takes place in the cecum and ascending colon, and very little in the transverse and descending colon, there is further absorption in the pelvic colon, which is responsible for the normal consistency of the feces. Considerable has also been written concerning the nature of the absorption and excretion of various substances by the colon, especially with reference to different concentrations of salts, sugar, heavy metals and drugs (4, 5, 6, 7, 8). Most of these investigations were made by means of isolated colon pouches, or previously purged colons, and sometimes with the animal under some type of anesthetic.

To our knowledge, little or no work has been done concerning the actual percentage differences in the water content of fecal material in the cecum, colon and rectum in apparently normal animals. Therefore, we undertook the investigation of this problem by determining the water content of the feces of the dog at different parts of the large intestine—namely, the cecum, proximal and distal colon, and rectum. Data will also be presented concerning the water content of the feces lodged in the distal colon and rectum when the contents of the small intestine are shunted directly into the distal colon. Finally we shall present a table showing how the water content of different parts of the human stool varies similarly to that of the dog.

Under anesthesia and by aseptic technique two fistulae were made in the colon of dogs, one in the cecum and another in the proximal colon, about 10 cm. distal to the ileo-cecal valve. The patency of the fistulae was maintained by a cannula made of a light weight hard rubber fiber about 4 cm. long, with an inside bore of 1.5 cm. The end inserted into the lumen of the colon had a flange of 1 cm. in width to insure retention of the cannula.

The muscular wall of the colon was ligated around the neck just above the flange. The other end of the cannula was brought to the outside through a stab wound in the abdominal wall, and was held in place by a rubber washer threaded on the cannula. The fecal material was prevented from entering the lumen of the cannula by a fiber plug of the same length and diameter as the inside bore of the cannula. This plug was held in place by a rubber screw cap. By means of these short cannulae we were able to take 2 or 3 gram samples of fecal material directly from the lumen of the cecum and colon by using a special curette. In later experiments we substituted for the hard rubber cannula a large pezzer catheter made of softer rubber, from which we cut the lower half of the bulb, the remaining half serving as a flange. This type of tube proved much more satisfactory. The permanent fistula devised by Mann and Bollman (9) in which a piece of reversed small intestine about 8 inches long is connected between the colon and abdominal wall, was also tried; this we found unsatisfactory, because a true sample of concentrated fecal material could not be obtained.

Each animal was given careful post-operative care, and after a week or ten days the colon had apparently regained its normal tone and function. The dogs were healthy and active, and on a standard diet of meat, bread and milk, passed stools of normal consistency. After the dogs had reached this point, we collected samples almost every morning from the cecum and proximal colon through the two fistulae, directly into previously weighed weighing bottles. Shortly after the samples had been collected, the dogs were allowed to exercise, and with surprising regularity they passed stools from 6 to 8 inches long within 15 minutes. We then collected 5 to 10 gram samples from the two ends and middle part of this stool, assuming that this material had just previously been in contact with the mucosa of the rectum, pelvic colon, and possibly the descending colon (Hurst (3) reports that upon defecation the colon may at times be emptied as high as the splenic flexure). All these samples were dried to a constant weight of 100°C. and the percentage water content calculated.

Hurst (3) and other early investigators believed, and physiology textbooks assert, that feces do not accumulate in the rectum until shortly before the desire to defecate. The opposite view is upheld by Alvarez (10) and other clinicians who find that the rectum is full of feces long before there is a desire to defecate. This fact was confirmed on different occasions by roentgenologic studies when after a barium meal the rectum of the dog, as well as that of man, was found to be full several hours before defecation took place. We also found that the first 2 inches of the stool were far more concentrated than the rest of the stool—this concentrated portion corresponds to the approximate length of the dog's rectum.

The average percentage water content of the samples from the different parts of the large intestine in one particular dog is presented in figure 1.

It was found that the average water content of the cecal samples was 87 per cent, while that of the proximal colon samples, 10 cm. distal to the ileocecal valve, was 10 per cent less. This difference clearly indicates that a considerable concentration of fecal material takes place in the cecum. The samples taken from the end and middle sections of the stool, those in contact with the descending and pelvic colon respectively, show a difference in concentration of only 4 per cent from the proximal colon sample—a very

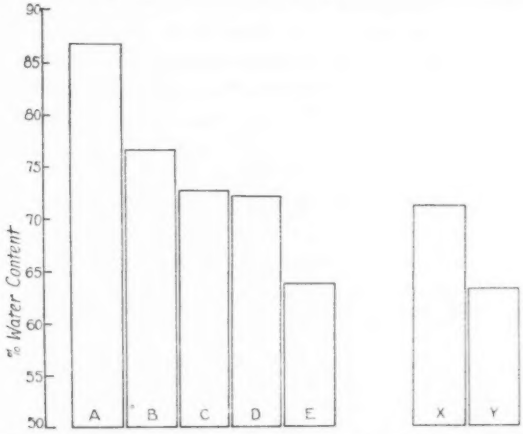

Fig. 1. Percentage water content of the fecal material in the colon of the dog.

A. Average percentage water content of 10 samples of fecal material collected from the cecal fistulae.

B. Average percentage water content of 9 samples of fecal material collected from the fistulae in the proximal colon.

C. Average percentage water content of 13 samples taken from the end portion of the stool.

D. Average percentage water content of 10 samples taken from the middle portion of the stool.

E. Average percentage water content of 13 samples taken from the rectal portion of the stool.

X.Y. Average percentage water content of 26 samples taken from the last and rectal ends of the stool, after the small intestine was anastomosed to the lower colon.

The percentage water content of all the samples in this curve was collected from the same animal.

slight one considering the length of the colon traversed. The sample taken from the first section of the stool, however, that in contact with the rectal mucosa, shows another marked water loss of nearly 10 per cent.

These results indicate that the rectum and cecum play a major part in the concentration of fecal material. This may be explained by the fact that the cecal and rectal portion of the large intestine have a much richer blood supply than the remaining part of the colon—a fact which we con-

firmed by actually outlining the vascular supply to the large intestine with injection material immediately after death from hemorrhage.

Considering the nature of these results, we were interested to learn the effects of shunting the contents of the small intestine directly into the descending colon, and thereby preventing the cecum from performing its normal part in the concentration of the feces. A second operation was therefore performed on the same animal, in which the ileum was sectioned near the ileocecal valve and closed. The ileum was then anastomosed laterally to the descending colon about 8 cm. below the splenic flexure. After the recovery of the dog, using the same diet as before, we again collected and dried samples of the stools, from the rectal and colon ends only, since the middle was so nearly like the colon end. We found that the difference in water content in the two ends of these stools was practically identical with the percentage difference in those collected from the two ends before the operation (see figure 1). At first we thought the rectum must be responsible for the main part of this concentration. However, it happened in the course of our experiments that we had one dog in which the cecal cannula was retained for four weeks after the ileum was anastomosed to the descending colon, so that in this dog we were able to continue the collection of cecal samples; and we found that the fecal material had in some manner been pushed back through the proximal colon into the cecum, where normal absorption could take place. We confirmed this on several occasions by adding carmine dye to the afternoon feeding of the dog. The next morning the dye showed up very distinctly in the cecal samples collected. Two days later no trace of carmine dye could be found

TABLE 1

Water content of samples of human feces

NUMBER	NAME	DATE	RECTAL	MIDDLE	END
1	F. R. S.	Oct. 31	69.77	81.34	82.00
2	F. R. S.	Nov. 1	65.33	78.08	78.24
3	F. R. S.	Nov. 4	71.12	82.79	81.13
4	F. R. S.	Nov. 5	72.55	85.31	85.13
5	F. R. S.	Nov. 7	72.25	79.96	82.03
6	F. R. S.	Nov. 8	71.50	82.04	84.17
7	F. R. S.	Nov. 10	74.81	82.34	85.58
Average			71.05	81.69	82.61
H. H. M.	Average (5 stools)		62.60	73.18	72.24
C. D.	Average (6 stools)		72.56	80.03	82.24
D. P.	Average (6 stools)		64.81	71.63	72.40

in the samples. This might indicate that some anti-peristaltic force tends to push fecal material back into the cecal region for concentration (11, 12, 13). On the other hand, the forward movement of the small intestine, combined with the pressure of the fecal material, might cause such an accumulation without the aid of anti-peristalsis.

Although it is common knowledge to anyone familiar with analyzing the human stool that the portion considered to have been in contact with the rectum is more solid than the rest of the stool, it was of interest to find the percentage difference in water content of the human stool, and whether the rectum has the power of concentrating the feces as it has in the dog. Five to seven stools were collected from four different persons, and samples were taken from the first, middle, and last portion of each stool and dried to constant weight in previously weighed weighing bottles (table 1).

The results proved to be very similar to those found in the dog; the first portion, which we assumed on the basis of previous roentgenologic studies to be in contact with the walls of the rectum was about three inches long (which is the approximate length of the rectum) and was about 7 to 12 per cent more concentrated than the portion just proximal to it. The difference between the middle and proximal portions was very slight—about 1 per cent.

It should be pointed out that all this work on both human and dog feces was carried out when no sign of constipation or diarrhea was evident.

CONCLUSIONS

1. The percentage water content of fecal material at points along the entire colon was determined. It was found that the feces in the proximal colon are 10 per cent more concentrated than that in the cecum, and that the rectal end of the stool is 10 per cent more concentrated than the part lodged in the lower colon.

2. There is a direct relation between the concentration of feces in the cecum and rectum and the blood supply to these parts.

3. When the contents of the small intestine are emptied directly into the descending colon by means of ileo-colostomy, there is evidence of some force which pushes the fecal material back into the cecum where it is concentrated as it was before the operation.

4. The percentage difference in the water content of the various parts of the human stool is similar to the percentage difference in that of the dog.

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CARBOHYDRATE CHANGES DURING RECOVERY FROM MUSCULAR CONTRACTION

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Skeletal muscle has two mechanisms available for disposing of the lactic acid that accumulates during contraction. One is the well known reaction described by Meyerhof (11), by which the major part of the lactic acid is converted to glycogen within the muscle at the expense of the oxidation of the smaller part to carbon dioxide and water. This reaction has been demonstrated by Meyerhof in isolated frog muscle, and has been shown to take place in isolated dog muscle by Shorr, Loebel and Richardson (16), and in isolated rabbit and beef muscle by Boyland (1).

The other mechanism involves the muscle-liver cycle of lactic acid and glucose first proposed by Himwich, Koskoff and Nahum (8). According to this, the lactic acid of the muscle diffuses into the blood stream and is removed from the blood by the liver, where it is converted to glucose which is in turn carried to the muscles and there converted to glycogen.

It becomes of considerable importance, in connection with theories of the source of energy for contraction, to ascertain the relative quantitative importance of these two mechanisms. If the "Meyerhof cycle" is shown to be the predominant one, then the findings of Owles (13), and of Margaria, Edwards and Dill (10), among others, that in light or moderate exercise little or no lactic acid appears in the blood, is easily explained on the basis of either the original Hill-Meyerhof theory or that of Lundsgaard. However, if it can be shown that the muscle-liver cycle predominates, then the findings can be explained only on the basis that the energy-yielding reactions are primarily oxidative, that the appearance of lactic acid in the blood during exercise is evidence of inadequate oxygen supply, and that the anaerobic mechanisms are but stop-gaps during the period of circulatory adjustment.

Eggleton and Evans (5) have shown that large amounts of lactic acid do enter the blood stream within a very short time after periods of violent muscular contraction, that the removal of this lactic acid is a slow process, and that the liver is responsible for the removal of the major part of the lactic acid. They state that lactic acid enters the blood stream from the

stimulated muscles throughout the entire recovery period. Although this work tends to show that the cycle involving the liver is the more important one, the nature of their experimental procedures was such that it was impossible to evaluate the quantitative importance of this mechanism relative to the "Meyerhof cycle" within the muscle.

The present work was undertaken to determine the relative importance of the two mechanisms. The procedure used is based on the following propositions: if, in a group of animals of one species which are of uniform age and weight, a certain muscle is stimulated isometrically for a definite, constant time, the amounts of lactic acid and of hexosephosphate formed, and of glycogen lost, should lie within a relatively narrow range. Now, if in another group of animals, similar to the first, the same muscle is stimulated under the same conditions, it would be expected that the same quantities of lactic acid and hexosephosphate would be formed, and glycogen lost, as in the first series. If the muscles in the second group are not analyzed at the end of the stimulation period, but are allowed to recover for a definite time and then submitted to analysis, the differences in the average values for the two groups can be held to give a reasonably accurate picture of the changes which have taken place during the recovery period. If it is found that the lactic acid loss from the muscle during the recovery period is greater than the net loss of total carbohydrate, this would show that a large part of the lactic acid had been converted to glycogen within the muscle. On the other hand, if the net loss of glycogen in the recovery period approaches the loss of lactic acid, it would show that diffusion into the blood stream accounted for the removal of the major part of the lactic acid. Of course, this does not permit any differentiation to be made between the lactic acid lost by oxidation and that lost by diffusion. However, it will be seen from the data that the changes are of such magnitude that except toward the end of the recovery period, there can be no doubt that oxidation could be of only minor quantitative importance in the removal of lactic acid.

The experiments were performed on rabbits which were about 3 months old and weighed from 1250 to 1500 grams. Under amytal-ether anesthesia, one gastrocnemius muscle was tetanized isometrically for 30 seconds, care being taken to leave the blood and nerve supply intact. The recovery periods used were 5, 10, 20 and 30 minutes. The muscles which were not allowed to recover were frozen within a few seconds after the end of the stimulation period. The resting muscle was frozen as soon as possible after the one which had been stimulated, generally within one minute. Determinations of lactic acid content, hexosephosphate formation and phosphocreatine hydrolysis were made by the methods used previously (14). Glycogen was determined by digestion with 30 per cent KOH, precipitation according to the method of Good, Kramer and Somogyi (7),

TABLE 1

Phosphorus, lactic acid and carbohydrate changes in muscles during recovery from isometric tetanus of 30 seconds' duration

Values as milligrams per cent of P, and of lactic acid and glycogen

RESTING MUSCLE				STIMULATED MUSCLE				DIFFERENCE			
Inorganic P	Inorganic plus phosphocreatine P	Lactic acid	Glycogen	Inorganic P	Inorganic plus phosphocreatine P	Lactic acid	Glycogen	Phosphocreatine hydrolysis	Hexosephosphate formation	Lactic acid	CARBOHYDRATE BALANCE
No recovery											
14	96	30	825	33	60	197	470	19	36	167	+21
18	95	20	850	28	68	180	480	10	27	160	−53
19	102	20	480	39	75	192	245	20	27	172	+94
21	90	17	980	34	60	150	605	13	30	133	−68
17	85	20	890	35	56	179	490	18	29	159	−73
19	102	16	1405	34	71	177	1000	15	31	161	−64
Average								16	30	159	−24
5 minutes' recovery											
14	92	29	1035	14	71	70	665	0	21	41	−207
19	94	18	840	17	81	73	610	−2	13	55	−100
20	95	20	1110	14	70	104	760	−6	25	84	−121
23	102	14	785	18	78	84	465	−5	24	70	−111
23	95	19	750	13	63	122	430	−10	32	103	−31
21	100	17	855	18	76	91	360	−3	24	74	−282
Average								−4	23	71	−142
Carbohydrate loss								142			
Lactic acid loss								88			
10 minutes' recovery											
19	99	14	935	13	80	69	625	−6	19	55	−145
19	99	28	1070	11	85	57	845	−8	14	29	−115
17	103	28	1045	12	77	84	705	−5	26	56	−133
18	97	17	780	13	78	54	450	−5	19	37	−183
16	100	20	835	11	81	63	450	−5	19	43	−232
Average								−6	19	45	−162
Carbohydrate loss								162			
Lactic acid loss								114			

TABLE 1—*Concluded*

RESTING MUSCLE				STIMULATED MUSCLE				DIFFERENCE			CARBOHYDRATE BALANCE
Inorganic P	Inorganic plus phosphocreatine P	Lactic acid	Glycogen	Inorganic P	Inorganic plus phosphocreatine P	Lactic acid	Glycogen	Phosphocreatine hydrolysis	Hexosephosphate fromation	Lactic acid	
20 minutes' recovery											
17	96	25	645	18	95	33	370	1	1	8	−261
20	97	18	980	17	90	38	660	−3	7	20	−260
20	100	19	1270	16	88	50	1025	−4	12	31	−144
14	85	37	915	14	79	58	610	0	6	19	−249
15	104	25	620	13	88	53	265	−2	16	28	−233
Average								−2	8	21	−229
		Carbohydrate loss					229				
		Lactic acid loss					138				
30 minutes' recovery											
17	92	22	520	17	87	26	265	0	5	4	−212
18	106	23	920	17	106	33	605	−1	0	10	−305
20	95	19	745	20	95	27	480	0	0	8	−257
19	90	13	655	17	79	20	545	−2	11	7	−39
Average								−1	4	7	−203
		Carbohydrate loss					203				
		Lactic acid loss					152				

hydrolysis of the precipitated glycogen with N/2 HCl, and determination of the reducing value of the neutralized hydrolysate with the Shaffer-Somogyi reagent 50 (15). Glycogen values in the tables are in terms of glucose equivalant.

There is a distinct advantage in stimulating a single muscle rather than the entire musculature of both hind legs, as was done by Eggleton and Evans. As the total quantity of lactic acid formed under the present conditions is small, it can be considered to be diffusing into blood which has a constant lactic acid content throughout the entire recovery period, for the load placed on the liver in removing this small amount of lactic acid is a light one.

Carbohydrate balances were drawn up in this way: For the resting muscle the sum of the lactic acid and glycogen contents was taken; for the stimulated muscle there was added to the sum of these values the glucose equivalent of the hexosephosphate difference between the two muscles. This glucose equivalent is obtained by multiplying the difference

between the sums of inorganic- and phosphocreatine-P of the resting and stimulated muscles by 5.8, the ratio between the equivalent weights of glucose and P.

Examination of the data shows that, while variations in the total carbohydrate content do appear between the two corresponding muscles of a single animal, the differences tend to disappear if several animals are considered together. In the group with no recovery the average net loss of carbohydrate is only 24 mgm. per cent. Although this small difference may be within the limits of experimental error, there are good reasons for regarding it as significant. For one thing, Cori, Closs and Cori (2) have shown that a certain amount of glucose is formed during a tetanus, the probable source of which is glycogen. Also, it is not unlikely that a small amount of lactic acid does diffuse into the blood stream during the tetanus. Both of these processes would result in an apparent net loss of carbohydrate in the present experiments, since muscle sugar determinations were not made.

During the first five minutes of recovery it is seen that a very considerable part of the lactic acid is lost from the muscle. On the basis of the "Meyerhof cycle" some part of this lactic acid should have been converted to glycogen within the muscle and the net loss of carbohydrate should be less than the loss of lactic acid. However, there is an additional net loss of carbohydrate over and above the decrease in lactic acid content. Throughout the entire recovery period, until the lactic acid content returns practically to the resting level, this condition persists; the loss in total carbohydrate is always greater than the loss of lactic acid.

It thus becomes evident that there is no detectable conversion of lactic acid to glycogen within the muscle recovering from contraction. It is thus seen that diffusion into the blood stream is the mechanism utilized by the muscle for disposing of lactic acid not oxidized. The "Meyerhof cycle" cannot be considered to play any appreciable rôle in the recovery process in mammalian muscle. Insofar as lactic acid removal is concerned, the liver must be regarded as the organ which accomplishes recovery from anaerobic work.

It is necessary to account for the extra loss of carbohydrate which becomes evident early in the recovery period. The phenomenon of "delayed lactic acid formation" can account, doubtless, for a considerable part of this extra carbohydrate loss. The lactic acid formed in this way also diffuses into the blood and the actual quantity so lost is greater than the observed difference in lactic acid content between the stimulated and recovering muscles. In addition, muscle is known to have an increased oxygen consumption above the resting rate during the period of recovery after contraction. The oxidation of carbohydrate by this extra oxygen probably accounts for the remaining portion of the loss.

There has been evidence available for some years that mammalian muscles do not convert injected lactic acid to glycogen. Elias and Schubert (6) found as early as 1918 that the intra-arterial injection of lactic acid did not result in glycogen deposition in the muscles of dogs. Janssen and Jost (9) were unable to find any evidence of glycogen deposition in the muscles following intravenous injection of lactic acid. It is only in isolated muscles that the "Meyerhof cycle" has been found to take place. In this connection it may be pointed out that Eggleton and Evans were unable to confirm the findings of Meyerhof, Lohmann and Meier (12), that perfusion of frog muscles with solutions containing lactate leads to deposition of glycogen.

The resynthesis of glycogen from hexosephosphate seems to proceed at a rather constant rate. Cori and Cori (3) found in experiments on rats that about 1 mgm. per cent of hexosephosphate-P per minute was converted to glycogen during the recovery period. This is approximately the rate found in the present experiments during the early part of the recovery period. The rate does apparently fall off later on. This falling off may be considered as mass action effect. As the concentration of hexosephosphate present decreases, the rate at which it is converted to glycogen could be expected to decrease. With regard to the path by which this substance is converted to glycogen, the suggestion is made by Cori and Cori that the immediate fate is conversion to lactic acid which then undergoes the Meyerhof reaction. They have shown that under anaerobic conditions hexosephosphate is converted to lactic acid, but it is evident from what has been said above that this reaction does not take place in the presence of oxygen, for any lactic acid so formed would escape into the blood and be lost to the muscles. Hence the conclusion is drawn that hexosephosphate is converted directly to glycogen by the reversal of the reactions leading to the formation thereof.

Examination of the values for inorganic phosphate in the muscles which recovered for 5 or 10 minutes shows that they are definitely lower than those for the corresponding resting muscles. This finding bears out the interpretation previously placed upon the function of the hydrolytic reaction of phosphocreatine (14). During the first 10 minutes of recovery the loss of lactic acid by the muscle is greater than the loss of base resulting from resynthesis of the phosphocreatine which had been hydrolyzed or converted to hexosephosphate during contraction. Hence the pH of the muscle fiber would be shifted to the alkaline side, were not some mechanism employed to remove additional base. The formation of phosphocreatine from some of the P which is normally present as inorganic phosphate would result in such a removal of base. The creatine necessary would be furnished by that liberated in the formation of hexosephosphate during contraction. Then, toward the end of the recovery period, as base is removed

by the conversion of hexosephosphate to phosphocreatine in greater measure than lactic acid is removed, this extra phosphocreatine is hydrolyzed. It is inadvisable to attempt any quantitative treatment of these data, but the shifts between inorganic phosphate and phosphocreatine that take place during contraction and recovery are invariably in the direction indicated by the requirement for maintaining a constant pH within the muscle fiber.

The question may properly be raised whether amytal or ether interferes with the resynthesis of muscle glycogen. There is no very great probability that they do. Eggleton and Evans found no difference between amytalization and decerebration with regard to the rate at which the muscles accumulate glycogen during the recovery period, and Debois (4) obtained resynthesis at approximately the same rate under ether that Eggleton and Evans did under amytal and in decerebrate animals. Since neither drug by itself interferes with this process when it is the sole anesthetic, there is no reason to assume that the two together will have such an effect.

The data presented here offer a satisfactory explanation of the findings of Owles and of Margaria, Edwards and Dill that in light or moderate exercise no lactic acid appears in the blood. Under their conditions the oxygen transporting mechanism was adequate to meet the demands placed upon it; the work performed was done by means of oxidative reactions and no appreciable amount of lactic acid was formed in the muscles. If the rate of work is such that the circulatory adjustment can be accomplished rapidly and completely, the small amount of lactic acid formed and not oxidized would be so rapidly disposed of by the liver that it would not be detected; only if the circulatory adjustment lags will there be any accumulation of lactic acid in the blood.

SUMMARY AND CONCLUSIONS

1. The entire amount of lactic acid formed during anaerobic activity in muscle diffuses into the blood stream during recovery.

2. Glycogen is not formed in the muscle during recovery from any of the lactic acid produced in anaerobic activity.

3. The "Meyerhof cycle" is shown to be of no significance in recovering mammalian muscle.

4. Hexosephosphate which is formed during contraction is resynthesized directly to glycogen during recovery, without intermediate breakdown to lactic acid.

5. The rate at which hexosephosphate is resynthesized to glycogen is practically constant throughout the recovery period.

6. Additional evidence is presented that the sole function of phosphocreatine hydrolysis in muscle is the maintenance of constant pH within the fiber.

7. The data confirm the theory that the reactions which yield energy for muscular activity are primarily oxidative.

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